AZD-3043

A Novel, Metabolically Labile Sedative–Hypnotic Agent with Rapid and Predictable Emergence from Hypnosis

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ABSTRACT

Background: Propofol can be associated with delayed awakening after prolonged infusion. The aim of this study was to characterize the preclinical pharmacology of AZD-3043, a positive allosteric modulator of the γ-aminobutyric acid type A (GABA_A) receptor containing a metabolically labile ester moiety. The authors postulated that its metabolic pathway would result in a short-acting clinical profile.

Methods: The effects of AZD-3043, propofol, and propadiod were studied on GABA_A receptor-mediated chloride currents in embryonic rat cortical neurons. Radioligand binding studies were also performed. The in vitro stability of AZD-3043 in whole blood and liver microsomes was evaluated. The duration of the loss of righting reflex and effects on the electroencephalograph evoked by bolus or infusion intravenous administration were assessed in rats. A mixed-effects kinetic-dynamic model using minipigs permitted exploration of the clinical pharmacology of AZD-3043.

Results: AZD-3043 potentiated GABA_A receptor-mediated chloride currents and inhibited [35S]tert-butylbiclophosphorothionate binding to GABA_A receptors. AZD-3043 was rapidly hydrolyzed in liver microsomes from humans and animals. AZD-3043 produced hypnosis and electroencephalograph depression in rats. Compared with propofol, AZD-3043 was shorter acting in rats and pigs. Computer simulation using the porcine kinetic-dynamic model demonstrated that AZD-3043 has very short 50 and 80% decrement times independent of infusion duration.

Conclusions: AZD-3043 is a positive allosteric modulator of the GABA_A receptor in vitro and a sedative–hypnotic agent in vivo. The ester depend metabolic pathway results in rapid clearance and short duration of action even for long infusions. AZD-3043 may have clinical potential as a sedative–hypnotic agent with rapid and predictable recovery.

What We Already Know about This Topic

• AZD-3043 is a chemical analog of propandid that was designed to be a rapidly metabolized hypnotic (“soft drug”) because of its ester moiety

What This Article Tells Us That Is New

• AZD-3043 is metabolized rapidly by liver microsomes
• It is a positive allosteric modulator of γ-aminobutyric acid_A-mediated chloride currents
• It produces rapid onset, dose-dependent electroencephalograph activity suppression in rats and pigs
• Emergence from AZD-3043-induced hypnosis is rapid and relatively unaffected by dose and infusion duration

The sedative–hypnotic activity of barbiturates, benzodiazepines, neurosteroids, and propofol (2,6-diisopropylphenol in lipid emulsion) results from potentiation of γ-aminobutyric acid (GABA)-mediated inhibition of synaptic activity within the central nervous system (CNS) via an allosteric interaction at the γ-aminobutyric acid type A (GABA_A) receptors. Thus, propofol produces a marked increase in the affinity of [3H]GABA binding to rat cortical membrane preparations and potentiates radiolabeled chlo-

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AZD-3043 Preclinical Summary

Fig. 1. The chemical structures of AZD-3043; its major inactive carboxylate metabolite, THRX-108893; and propanidid are shown. AZD-3043 is an analog of propanidid that is substituted with an aryl ethoxy, rather than an aryl methoxy group. In rodents, THRX-108893 accounts for more than 90% of the total radioactivity in urine, feces, plasma, liver, and kidney after dosing with radiolabeled AZD-3043.

Ride uptake evoked by muscimol, a GABA<sub>A</sub> receptor agonist. Acting at a binding site distinct from that of the barbiturates, benzodiazepines, or neurosteroid sedative–hypnotics, propofol increases the open probability of GABA<sub>A</sub> receptor chloride channels. Propofol has gained considerable use for the induction and maintenance of sedation–hypnosis because patient awakening is relatively rapid. However, the rapidity of emergence from propofol-mediated sedation–hypnosis is dose-dependent. To avoid tissue accumulation and delayed or unpredictable patient emergence, dose titration is necessary, particularly with prolonged infusion.

Propanidid ([4-diethylcarbamoylmethoxy-3-methoxy-phenyl]acetic acid propyl ester) is a short-acting sedative–hypnotic agent containing an ester moiety that was available in some countries in the 1960s and 1970s. As was the case with propofol, propanidid was introduced as a solution containing Cremophor® (BASF Corporation, Florham Park, NJ); both were associated with histamine release and adverse hemodynamic effects. Unlike the situation with propofol, propanidid was not reintroduced commercially in an alternative, more acceptable, lipid-based emulsion.

Like propanidid, AZD-3043 ([4-[[N,N-Diethylcarbamoyl]methoxy]-3-ethoxyphenyl]acetic acid propyl ester) contains a metabolically labile ester (fig. 1). Formerly known as TD-4756, AZD-3043 is formulated in a lipid emulsion similar to that used for propofol. It was postulated that emergence from hypnosis with AZD-3043 would be more rapid and predictable than that with propofol. This study characterized the nature of the interaction of AZD-3043 with the GABA<sub>A</sub> receptor in vitro and examined the hypnotic profile of the compound in vivo. The stability of AZD-3043 was also evaluated in vitro in liver microsomes and whole blood from several species, including man. The onset, duration of hypnosis, and recovery profile after intravenous (IV) bolus and infusion administration of AZD-3043 was evaluated in rats. The electroencephalogram of the rat was recorded during AZD-3043 administration. The pharmacokinetics and pharmacodynamics of AZD-3043 were investigated in a porcine model; pharmacokinetic simulations based on a combined kinetic-dynamic model were used to explore the clinical behavior of AZD-3043.

**Materials and Methods**

All experiments were conducted according to guidelines established by the Institutional Animal Care and Use Committee at Theravance, Inc. (South San Francisco, California), Brigham Young University (Provo, Utah) (rat electroencephalography studies), or the University of Utah (Salt Lake City, Utah) (porcine study). The procedures described complied with the Animal Welfare Act and Public Health Service Policy, 1999. Human blood was collected under a voluntary blood donor program at Theravance, Inc., the policy for which was set up by an occupational health physician and included volunteer consent and Institutional Biosafety Committee approval.

**In Vitro Electrophysiology**

Cultures of cortical neurons were prepared from embryos of untimed (approximately E-18) pregnant dams. Cells were seeded onto poly-D-lysine (100 µg/ml) coated glass coverslips in Dulbecco’s Modified Eagle’s Medium supplemented with 10% fetal bovine serum and 200 µg/ml penicillin-streptomycin. One day after plating, cells were maintained in serum-free Dulbecco’s Modified Eagle’s Medium, supplemented with B27 (Gibco® B-27; Life Technologies, Carlsbad, CA). Cultures were maintained in a humidified, 5% CO<sub>2</sub>, 95% O<sub>2</sub> environment at 37°C until use.

The coverslips were transferred to the recording chamber and perfused continuously (0.5 ml/min) with bathing solution at room temperature. The bathing solution (pH 7.4) contained (in mM): NaCl (140), KCl (5.4), CaCl<sub>2</sub> (1.3), glucose (33), HEPES (25), and 0.3 µM tetrodotoxin. Gigaohm seals were formed between the cell and a patch electrode (initial resistance 1–2 MΩ). The patch electrode contained (in mM): CsCl (140), CaCl<sub>2</sub> (1), EGTA (11), and HEPES (10) with pH 7.2 with CsOH. The membrane beneath the electrode tip was ruptured to establish a whole cell patch clamp configuration. Recording commenced in the voltage clamp mode, with an initial holding potential of −60 mV.

GABA (5 µM, the EC<sub>20</sub> concentration) was applied once every 2 min (Bio-Logic RSC-200 rapid solution exchanger; Bio-Logic SAS, Claye, France) for a total of 20 min per cell in the absence or presence of increasing concentrations of...
AZD-3043, propofol, or propanidid. The EC\textsubscript{50} for GABA was determined on a separate pool of primary cortical neurons. Within this control pool, the GABA-evoked responses were similar in potency (although varying in magnitude).

Ionic currents were recorded using an AXON Instruments AxoPatch 200B amplifier (Axon Instruments, Inc., Union City, CA). Peak current amplitudes were measured using pCLAMP® software (Molecular Devices Corporation, Sunnyvale, CA), and data were then exported to Origin® (OriginLab Corp., Northampton, MA), where the individual current amplitudes were normalized to the initial current amplitude in the absence of AZD-3043, propofol, or propanidid.

**Radioligand Binding Studies**

To investigate the agents’ potential off-target activities, radioligand binding experiments were performed in duplicate with AZD-3043 (50 μM), propanidid (50 μM), and their primary carboxylic metabolites at 30 different receptors. With the exception of the human β\textsubscript{1}- and β\textsubscript{2}-adrenoceptor and the muscarinic M\textsubscript{1–5} receptor subtype binding studies which were performed at Theravance, Inc., investigations were carried out under contract at Cerep (Celle l’Evescault, France; see catalog for assay conditions). Nonspecific radioligand binding was determined in the presence of an excess of unlabeled ligand, and specific binding was defined as the difference between total and nonspecific binding. Data (individual values and means) were expressed as a percentage inhibition of specific binding.

**Stability in Blood and Liver Microsomes**

Human whole blood stability experiments were conducted in whole blood from seven separate human donors. Single experiments were conducted with pooled blood (in duplicate) from rats (n = 5), cats (n = 4), beagle dogs (n = 4), and minipigs (n = 4). Blood was collected in Vacutainer™ (Becton, Dickinson and Company, Franklin Lakes, NJ) tubes containing sodium heparin. Single experiments were conducted with pooled liver microsomes (in duplicate) from beagle dogs (n = 4), cynomolgus monkeys (n = 8), humans (n = 10), and minipigs (n = 3) purchased from Xenotech, Inc. (Kansas City, KS). *In vitro* stability of AZD-3043 (50 μM) was evaluated after the samples were incubated (for 10 s to 60 min) with whole blood or liver microsomes (1 mg protein/ml, 37°C) in polypropylene test tubes. In experiments using blood, incubation samples (0.3 ml) were withdrawn and mixed with 0.6 ml ice-cold ethanol to terminate metabolism. The samples were centrifuged, and the supernatants dried under a stream of nitrogen at room temperature. The samples were centrifuged, and the supernatants (40 μl) from the blood studies or 50 μl from microsomal experiments) were injected on to an Agilent 1100 high pressure liquid chromatography system (Agilent Technologies, Santa Clara, CA) equipped with a Luna C18 analytical column (5 μm, 150 × 2.0 mm; Phenomenex, Torrance, CA) at a flow rate of 0.5 ml/min. The mobile phases were acetonitrile, 5%, in trifluoroacetic acid, 0.1% (Mobile Phase A), and acetonitrile, 95%, in trifluoroacetic acid, 0.1%, (Mobile Phase B), and a wavelength of 214 nm was used. The mobile phase gradient started at 94% A/6% B and ramped to 35% A/65% B over 15 min before reverting to an isocratic hold at 94% A/6% B over the final 5 min. The substrate remaining at each sampling time point was monitored by peak area on the chromatogram and expressed as a percentage of that at time 0. The observed rate constant (k\textsubscript{obs}) for substrate disappearance was determined as the slope of the line from the plot of the natural log of the percentage of substrate remaining *versus* time of incubation. The half-life (t\textsubscript{1/2}; *i.e.*, the time required for 50% of the substrate to be metabolized) was calculated according to the following equation: T\textsubscript{1/2} = 0.693/k\textsubscript{obs}.

**Rodent Pharmacology**

Adult male and female Sprague-Dawley rats (Harlan, Indianapolis, IN) weighing 120–750 g were used. Food and water were available *ad libitum*.

**Hypnosis in Response to Bolus Administration**

A rodent model\textsuperscript{8} was used to provide a measure of onset and duration of hypnosis and to evaluate the recovery profile after return of consciousness. AZD-3043 (6.5–27 mg/kg), propofol (5–20 mg/kg), propanidid (15–45 mg/kg), and their respective vehicles were administered to rats *via* the tail vein (1 ml/kg over 5 s). The hypnotic activities of THRX-108893 (0.2–0.9 g/kg) and 1-propanol (0.1–0.5 g/kg), the primary metabolites of AZD-3043, were also assessed. In one study, remifentanil (2.5 and 10 μg/kg) or fentanyl (10 μg/kg) was coadministered with AZD-3043, propofol, or propanidid. After the doses were given, rats were placed in a supine position on a heating blanket to maintain body temperature at 37°C–38°C (monitored rectally with a sensor [Physitemp BAT-12; Physitemp Instruments, Inc., Clifton, NJ]). The duration of the loss of the righting reflex was recorded. Upon regaining their righting reflex, the time that lapsed until the rats were able to grip and climb a steel frame and ambulate normally was determined (the “time to behavioral recovery”). To provide a measure of relative potencies, the dose estimated to produce a mean loss of righting reflex of 2 min (*i.e.*, the “2-min bolus dose”) was determined from the dose-response curves. In one study, the ED\textsubscript{50} values for the loss of righting reflex produced by AZD-3043 and propofol were determined (*i.e.*, the dose at which 50% of rats lost their righting reflex, regardless of duration). The ED\textsubscript{50} values were derived using Prism graphics software 3.0 (GraphPad, Inc., San Diego, CA).
Hypnosis in Response to IV Infusion

Induction of hypnosis in rats was achieved using the approximate 2-min bolus dose of each compound, and immediately after induction, infusion, via the tail vein, was commenced at one half that dose per minute. In some experiments, the infusion rate was maintained for 20 min. In other studies, the infusion rate was modified to ensure that the depth of hypnosis remained constant, as monitored by the magnitude of a withdrawal reflex to intermittent paw pinch provided by a pair of forceps. The degree of closure of the tips of the forceps was fixed to ensure that a consistent paw pressure was applied. After completion of the infusion (20 min, or 3 or 5 h later), the duration of the loss of righting reflex and time to behavioral recovery were determined.

Rodent Electroencephalography

With the animals anesthetized (40 mg/kg intraperitoneal pentobarbital and 20–27 mg/kg intramuscular ketamine), a longitudinal incision was made in the rat’s neck, and a jugular vein was catheterized. After placement of the rat in a stereotaxic frame, a midline incision (2 cm in length) was made in the scalp. Holes (1–2 mm in diameter) were drilled in seven perimeter skull locations for insertion of grounding and support screws. A silver grounding wire was wrapped around the seven supporting skull screws. Holes (1 mm in diameter) were drilled in four skull locations, corresponding to the bilateral parietal and frontal cortices. Four stainless steel skull screws, wrapped with 120 μm heavy polyimide enamel-coated stainless steel wire, served as the electroencephalography electrodes. The ground and electroencephalography electrodes were connected to a subminiature Amphenol nine-pin D-connector (Amphenol Corporation, Cedar Grove, NJ) cemented to the cranium with dental acrylic.

At least 1 week after surgery, each rat was placed in a sound-proof box. Electroencephalography activity was amplified (×100–10,000) and filtered (0.1–100 Hz) using a multichannel signal conditioner (National Instruments, Austin, TX). After baseline data were collected for 20 min, rats were dosed via the jugular catheter (1 ml/kg over 5 s) with vehicle, followed by increasing doses of AZD-3043 (5, 10, 20, and 30 mg/kg) or propofol (1, 3, 6, and 10 mg/kg) at 30-min intervals (n = 6 rats in a crossover design, 5-day washout between treatments). The electroencephalography data were digitized at 500 Hz and analysis performed as described previously.9 Epochs, consisting of 2 s of averaged electroencephalography data, were classified based on an “interpretation map” that was constructed from a learning algorithm of the electroencephalography data.10 Classification values represent the degree of presence (more than 0) or absence (less than 0) of a pattern of features (e.g., phase-weighted frequencies) inherent in the raw spontaneous electroencephalography. The learning algorithm identified feature patterns that discriminated optimally between drug and nondrug states. Five sets of frequencies were used to classify the electroencephalography epochs (Gaussian center frequency ± Gaussian half-width = 3.5 ± 3.5 Hz, 5 ± 5 Hz, 10.0 ± 10.0 Hz, 14.0 ± 14.0 Hz, and 17.0 ± 31.0 Hz). Using commercial signal processing software (NeuroInsight LLC, Bountiful, UT), statistical analysis (pattern discovery, recognition, and verification) was performed. Electroencephalography patterns before and after injection were compared to detect drug effects, and electroencephalography activation plots, representing 2 s of filtered electroencephalography activity, were created. The values in the electroencephalography activation plots ranged from −1 (fully inactivated) to 1 (fully activated).

Large Mammal Pharmacology

Minipigs were used to model the clinical pharmacokinetics and pharmacodynamics of AZD-3043. All animals were obtained from a single breeding source and were approximately the same age.

The anesthetic induction was performed using intramuscular injection of Telazol® (Wyeth, New York, NY), xylazine, and ketamine.10 Anesthesia was maintained with inhaled isoflurane. The pig’s trachea was intubated and mechanically ventilated to maintain arterial pCO2 at approximately 35–40 mmHg. Physiologic monitoring included the electrocardiogram, a pulmonary artery catheter, femoral arterial catheters, pulse oximetry (Spo2), and a urinary catheter.

AZD-3043 (1.5 or 3.0 mg · kg−1 · min−1, 20 min) was administered via the peripheral IV catheter. Arterial blood samples (1 ml each) were collected at 0 (control sample), 2, 4, 8, 12, 16, 20, 21, 22, 23, 24, 25, 27.5, 30, 35, 40, 45, 60, 90, 120, and 180 min after the start of the infusion. Immediately after collection, methanol (2.5 ml) was added to each blood sample (1 ml), and the mixture vortexed. Samples were then centrifuged (3,000 g) for 15 min at 5°C and 2 ml supernatant transferred to a new tube for analysis.

Blood concentrations of AZD-3043 and the acid metabolite were determined in minipigs by LC-MS/MS (Sciex API 3000; Applied Biosystems, Foster City, CA). Samples (25 μl) were injected on a Higgins Analytical C18 Targa column (30 × 0.5 mm; 5 μM) with a flow rate of 0.125 ml/min (Higgins Analytical Inc., Mountain View, CA). Mobile phase A consisted of 0.25% formic acid in water, and mobile phase B consisted of 0.25% formic acid in acetonitrile. The gradient elution started with a 1-min loading step at 30% B followed by a linear gradient to 80% B over 2 min, a 95% B wash for 30 s, and a 1-min reequilibration at 10% B. The mass spectrometer was operated in positive ion multiple reaction monitoring mode. The calibration range of the assay was from 0.01 μg/ml (limit of quantification) to 1,000 μg/ml in whole blood.

The raw electroencephalography was collected using bipolar, frontal, low impedance surface electrodes and processed into the Bispectral Index (BIS® monitor; Aspect Medical Systems, Natick, MA) parameter. The BIS was used as the primary pharmacodynamic signal.
Pharmacokinetic–Pharmacodynamic Analysis
The raw concentration versus time data were plotted and inspected. The pharmacokinetic parameters for a three-compartment model were estimated using a mixed-effects population approach based on the NONMEM program (University of California, San Francisco, CA) with a log-normal error model applied to interindividual error on each parameter. Model performance was assessed by visual inspection of residual plots and by computing the accuracy and bias of the model predictions as described by Varvel et al. The raw BIS versus time data were plotted and inspected. The pharmacodynamic parameters for an inhibitory sigmoidal E_max model with an effect compartment linking the plasma and the effect site were estimated using a naïve pooled approach implemented in NONMEM.

Computer Simulations
Computer simulations using the combined population pharmacokinetic–pharmacodynamic models were performed to provide an illustration of the predicted time course of AZD-3043 effect-site concentrations and BIS effect after the administration of clinically relevant doses of the drug. For comparison, similar simulations were performed for propofol using pharmacokinetic and pharmacodynamic parameters from the literature. We also performed 80 and 50% decrement time simulations, predicting the time necessary to achieve an 80 or 50% decrease in effect site concentration after termination of a variable-length, continuous, steady-state infusion. The simulations were implemented using PKPD Tools.†‡

Drug Formulations
AZD-3043, THRX-108893, and propanidid were synthesized at Theravance, Inc., whereas propofol (for in vitro studies) was purchased from Sigma Aldrich (St. Louis, MO). For in vitro electrophysiology, radioligand binding and blood-liver microsome stability studies, test compounds were formulated in dimethyl sulfoxide to provide stock solutions of 10, 1, or 20 μM, respectively, which were then diluted with perfusion buffer or distilled water. For in vivo studies, propofol (Diprivan®, 1%; Astra Zeneca, Caponago, Italy), 1-propanol (high pressure liquid chromatography grade; Sigma-Aldrich, Dallas, Texas), remifentanil (Ultiva®, Abbott Labs, North Chicago, IL), and fentanyl (fentanyl citrate; Baxter, Deerfield, IL) were purchased. AZD-3043 and propanidid were formulated in lipemusions, whereas THRX-108893 and 1-propanol were formulated in distilled water. Dilutions of AZD-3043, propropofol, propofol, THRX-108893, and 1-propanol were made in their respective vehicles. Dilutions of fentanyl and remifentanil were made in dextrose, 5%, in distilled water.

Statistical Analysis
In the rat hypnotic infusion experiments, two types of statistical analyses were performed. For the 20-min infusion studies to compare the emergence times of propofol, AZD-3043, and propanidid, a one-way ANOVA with Dunnett’s post hoc test (statistical significance at P < 0.05) was used. In a subsequent study in which the emergence times were analyzed at different infusion durations (i.e., 20 min, or 3 or 5 h), a Student unpaired t test (two-tailed hypothesis testing, with statistical significance at P < 0.05) was used to compare the effects of propofol and AZD-3043 at each of the time points. Data were analyzed using Prism (GraphPad, Inc.) software.

Results
In Vitro Electrophysiology
The vehicle, dimethyl sulfoxide, had no effect on the chloride currents recorded in embryonic rat cortical neurons. GABA (3 μM to 1 mM) and the selective GABA_A receptor agonist, muscimol (0.3 μM to 1 mM), evoked a concentration-dependent increase in chloride currents. The GABA_A receptor selective antagonist bicuculline (10 μM) inhibited the GABA-induced chloride currents, which were therefore concluded to be GABA_A receptor-mediated. AZD-3043, propanidid, and propofol potentiated the GABA (5 μM; EC20 concentration)-mediated current (EC50 values of 36, 26, and 6 μM, respectively; fig. 2, A and B). The maximum potentiation achieved by AZD-3043 was approximately 65% of that produced by propofol and 115% of that of propanidid. THRX-108893 (300 μM) had no effect on GABA-evoked current (data not shown).

Radioligand Binding Studies
Of the 30 binding sites at which they were tested, AZD-3043 and propanidid (each 50 μM) produced more than 50% inhibition of specific binding at GABA_A chloride channels (83 and 65%, respectively); see AZD-3043 data in Supplemental Digital Content 1, http://links.lww.com/ALN/A848, figure 1. Propanidid (50 μM) produced 49% inhibition of strychnine-sensitive glycine receptor binding, whereas AZD-3043 (50 μM) was inactive. The carboxylate metabolites of AZD-3043 and propanidid produced less than 50% inhibition of specific binding in all the radioligand binding assays at 200 μM; see THRX-108893 data in Supplemental Digital Content 1, http://links.lww.com/ALN/A848, figure 2.

Stability in Blood and Liver Microsomes
AZD-3043 was rapidly metabolized in human, minipig, dog, and cynomolgus monkey liver microsomes (t1/2 values in table 1). There was no difference in the metabolic stability of AZD-3043 in human liver microsomes in the absence or presence of a nicotinamide adenine dinucleotide phosphate-regenerating system, suggesting that the

metabolism of AZD-3043 is not mediated by cytochrome P450. Metabolism of AZD-3043 resulted in the formation of the carboxylate metabolite THRX-108893 in all species evaluated.

AZD-3043 was rapidly metabolized in rat and guinea pig whole blood (mean t½ values of 0.6 and 0.1 min, respectively) but more slowly metabolized in human blood (mean t½ ≈ 27 min). In cat, dog, or minipig blood, AZD-3043 was stable (table 1). The rank order of stability of AZD-3043 in blood from the various species tested was cat > dog > minipig > human > rat > guinea pig. When AZD-3043 was metabolized, the formation of THRX-108893 was observed.

Table 1. The In Vitro Stability of AZD-3043 in Various Species

<table>
<thead>
<tr>
<th>Preparation</th>
<th>Species</th>
<th>Mean t½ Value (min)</th>
</tr>
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<tbody>
<tr>
<td>Liver microsomes</td>
<td>Human</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Dog</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>Minipig</td>
<td>0.4</td>
</tr>
<tr>
<td></td>
<td>Cynomolgus monkey</td>
<td>3</td>
</tr>
<tr>
<td>Whole blood</td>
<td>Human</td>
<td>27 ± 8*</td>
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<tr>
<td></td>
<td>Cat</td>
<td>&gt;60</td>
</tr>
<tr>
<td></td>
<td>Dog</td>
<td>&gt;60</td>
</tr>
<tr>
<td></td>
<td>Minipig</td>
<td>&gt;60</td>
</tr>
<tr>
<td></td>
<td>Rat</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td>Guinea pig</td>
<td>0.1</td>
</tr>
</tbody>
</table>

Data are expressed with respect to the mean t½ values from at least two separate studies.

* Human whole blood stability experiments were conducted in whole blood from seven separate human donors.

Rodent Pharmacology
Hypnosis in Response to Bolus Administration. AZD-3043 (6.5, 13, and 27 mg/kg; n = 4 per dose), propanidid (15, 30, and 45 mg/kg; n = 4, 5, and 3, respectively), and propofol (5, 10, and 20 mg/kg; n = 9, 7, and 7, respectively), but not their vehicles, which were inactive (data not shown), produced a dose-dependent loss of the righting reflex after IV bolus administration (fig. 3A). For each compound, onset of hypnosis was equally rapid (less than 10 s from completion of injection). The slope of the AZD-3043 and propanidid dose-response curves was shallower than that of propofol. In addition, the time to behavioral recovery for AZD-3043 and propanidid, but not propofol, changed little with increasing doses (fig. 3B). THRX-108893 and 1-propanol, the primary metabolites of AZD-3043, were also evaluated to determine whether they possessed hypnotic activity. THRX-108893 (200–900 mg/kg; n = 2 per dose) failed to produce hypnosis, whereas 1-propanol (200–500 mg/kg; n = 3 or 4 per dose) was found to be only weakly active. With respect to the dose of each compound calculated to produce a mean loss of...
Analgesia was evident. Upon coadministration of fentanyl (10–20 mg · kg⁻¹ · min⁻¹; n = 4), propanidid (20 mg · kg⁻¹ · min⁻¹; n = 3), or propofol (3 · kg⁻¹ · min⁻¹; n = 5) in rats. D5W/H11005/H11005/H926210 produced by AZD-3043 (7.5 mg · kg⁻¹ · min⁻¹; n = 3), propanidid (20 mg · kg⁻¹ · min⁻¹; n = 3), or propofol (3 · kg⁻¹ · min⁻¹; n = 5) in rats. *P < 0.05 compared with propofol, one-way ANOVA and Dunnett’s post hoc test.

**Hypnosis in Response to IV Infusion.** After induction of hypnosis with the approximate 2-min bolus doses of AZD-3043, propanidid, or propofol (15, 30, and 6 mg/kg, respectively), hypnosis was maintained by a 20-min infusion at one half that dose · kg⁻¹ · min⁻¹. Upon constant infusion of AZD-3043 or propanidid, the depth of hypnosis remained at a similar level, as indicated by a consistent, and small, withdrawal response to intermittent noxious paw pinch with a pair of forceps. In contrast, the depth of hypnosis increased during the 20-min propofol infusion. After the infusion was completed, recovery of the righting reflex was rapid in rats treated with AZD-3043 or propanidid but prolonged and more variable in those that had received propofol (mean durations ± SD of 1.7 ± 0.2, 2.1 ± 0.9, and 72.1 ± 23.7 min, respectively; fig. 5A). Similarly, time to behavioral recovery (fig. 5B) was more rapid after AZD-3043 or propanidid infusion (mean durations ± SD of 1.1 ± 0.2 and 1.8 ± 0.7 min, respectively) compared with propofol (mean duration, 33.1 ± 10.7 min).

To evaluate further the hypnotic profile of AZD-3043, infusions of 20 min and 3 or 5 h duration were carried out, and the infusion rate was adjusted, when necessary, to maintain a consistent depth of hypnosis (a small withdrawal reflex to noxious paw pinch). The return of the righting reflex and time to behavioral recovery after termination of the infusion of AZD-3043, but not propofol, occurred rapidly and was relatively unaffected by the duration of infusion (fig. 6).

**Rat Electroencephalography.** The electroencephalography was recorded in rats to compare the effects of AZD-3043 and propofol on brain activity and their duration of action after bolus IV dosing. The electroencephalography analysis was able to detect modulation of electrocortical activity produced by AZD-3043 and propofol within 5–10 s after their administration. Administration of AZD-3043 (5–30 mg/kg) and propofol (1–10 mg/kg), but not their respective vehicles, produced a rapid, dose-dependent suppression of the rat electroencephalogram (10 μg/kg), the leftward shifts in the hypnosis dose-response curves of AZD-3043 and propofol were similar (fig. 4).

**Fig. 4.** Duration (mean ± SD) of the loss of righting reflex produced by AZD-3043 (A; n = 3, 4, or 5) and propofol (B; n = 3, 4, or 5) upon coadministration with remifentanil (2.5 or 10 μg/kg), fentanyl (10 μg/kg), or vehicle (D5W; 1 ml/kg) in rats. D5W = dextrose, 5%, in water.

**Fig. 5.** Duration (mean ± SD) of the loss of righting reflex (A) and time to behavioral recovery (B) after a 20-min infusion of AZD-3043 (7.5 mg · kg⁻¹ · min⁻¹; n = 4), propanidid (20 mg · kg⁻¹ · min⁻¹; n = 3), or propofol (3 · kg⁻¹ · min⁻¹; n = 5) in rats. *P < 0.05 compared with propofol, one-way ANOVA and Dunnett’s post hoc test.
gram; see AZD-3043 and propofol processed data and raw electroencephalogram tracings in Supplemental Digital Content 1, http://links.lww.com/ALN/A848, figures 3–16. Suppression of the electroencephalogram by AZD-3043 (5–30 mg/kg) was short-lived (recovery within approximately 5 min). Propofol produced a greater, dose-dependent increase in the duration of electroencephalographic suppression and decrease in the slope of the electroencephalogram recovery relative to AZD-3043 (see Supplemental Digital Content 1, http://links.lww.com/ALN/A848, figs. 3–16).

Large Mammal Pharmacology

All five minipigs completed the experiments. One male and four female swine, weighing 27.4–35.0 kg (average = 30.5 ± 3.3 kg) were used. No infusion was terminated early because of an adverse event, such as severe bradycardia, tachycardia, or hypotension.

Pharmacokinetic–Pharmacodynamic Analysis

Four data points of 105 were removed from the pharmacokinetic analysis because they were considered to be outliers due to a sample processing problem. Figure 7A shows the raw concentration-time curves for all animals.

The three-compartment model adequately described the pharmacokinetics of AZD-3043. The best model parameters along with the numerical measures of model performance are shown in table 2. In general terms, these models have excellent performance in terms of bias and accuracy.

Data from one animal was excluded from the pharmacodynamic analysis because of an inadequate electroencephalographic signal. Figure 7B shows the shapes of the raw concentration-effect relationships and the population estimate. The pharmacodynamics were well described by the model (model parameters displayed in table 2).

Computer Simulations

The simulations revealed substantial differences in the predicted clinical pharmacology behavior of AZD-3043 and propofol. The simulations of a combined bolus and infusion dosing scheme for the two drugs are displayed in figure 8A; these simulations predict a substantially faster drug offset for AZD-3043 compared with propofol. The decrement time simulations displayed in figure 8B illustrate several important points. First, the 50% decrement times showed moderate difference between two drugs for long infusions. Second, AZD-3043 exhibits a substantially shorter 80% decrement time than does propofol, especially after long infusion.

Discussion

Propofol is a positive allosteric modulator of the GABA<sub>A</sub> receptor and produces sedation–hypnosis by potentiating GABA<sub>A</sub>-mediated neuronal inhibition within the CNS.1,2,15 Clinically, dose titration of propofol is common, particularly upon prolonged infusion, to ensure acceptable emergence from sedation or hypnosis. AZD-3043 is a novel sedative–hypnotic agent that was designed to be hydrolyzed rapidly by esterases in liver or blood. The in vitro stability data in the current study demonstrated that AZD-3043 is metabolized...
The apparent lower intrinsic activity of AZD-3043 and propanidid but was less potent than propofol. The significance of the parallelism, AZD-3043 had a potency similar to that of propofol. The in vivo pharmacokinetic profile of AZD-3043 will be the subject of a future publication.

Whole cell patch clamp data confirmed that like propofol and propanidid, AZD-3043 potentiated GABA\(_A\)-mediated chloride currents. In the embryonic rat cortical neuron preparation, AZD-3043 had a potency similar to that of propanidid but was less potent than propofol. The significance of the apparent lower intrinsic activity of AZD-3043 and propanidid relative to propofol in these rat neurons is unclear. It is possible that AZD-3043 and propofol possess different efficacies and/or affinities for the many subtypes of GABA\(_A\) receptors in the CNS. Pentameric in structure, the GABA\(_A\) receptor consists of numerous combinations of different subunits (i.e., \(\alpha\), \(\beta\), \(\gamma\), \(\delta\), \(\varepsilon\), \(\theta\), and \(\pi\)), and although more than 10,000 pentameric subunit combinations are possible, it is postulated that fewer than 10 subtypes contribute to the major physiologic responses to GABA in the adult mammalian brain. It is also possible that the low aqueous solubilities of each test agent, despite the absence of any visible compound precipitation, contributed to the apparent differences noted in their intrinsic activities. In vivo, AZD-3043 and propofol produced a similar degree of electroencephalographic suppression in pigs, cats (unpublished observations; contemporaneous cat experiment data on file at Theravance, South San Francisco; the studies were conducted between September and December 2004, supervised by David T. Beattie, Ph.D., Theravance, Inc., South San Francisco, California).

### Table 2. Population Compartmental Pharmacokinetic Parameters by Mixed Effects Modeling and Pharmacodynamic Parameters by Naïve Pooled Analysis

<table>
<thead>
<tr>
<th>Pharmacokinetic parameters</th>
<th>Estimate</th>
<th>CV (%)</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volumes (l)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Central</td>
<td>2.84</td>
<td>24.7</td>
<td>2.17, 3.39</td>
</tr>
<tr>
<td>Peripheral 1</td>
<td>5.6</td>
<td>32.4</td>
<td>4.24, 7.62</td>
</tr>
<tr>
<td>Peripheral 2</td>
<td>3.37</td>
<td>34.1</td>
<td>3.31, 7.09</td>
</tr>
<tr>
<td>Clearances (/min)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Central</td>
<td>1.63</td>
<td>13.8</td>
<td>1.42, 1.86</td>
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<tr>
<td>Intercompartmental 1</td>
<td>0.15</td>
<td>36.7</td>
<td>0.10, 0.28</td>
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<tr>
<td>Intercompartmental 2</td>
<td>0.486</td>
<td>46.3</td>
<td>0.33, 0.69</td>
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<td>MDPE</td>
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</tr>
<tr>
<td>MDAPE</td>
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</table>

Pharmacodynamic parameters

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Estimate</th>
<th>CV (%)</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>(E_0)</td>
<td>84.6</td>
<td></td>
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</tr>
<tr>
<td>(E_{\max})</td>
<td>0.705</td>
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<tr>
<td>(C_{E50}) ((\mu)/ml)</td>
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<tr>
<td>(\gamma)</td>
<td>4.42</td>
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</tr>
<tr>
<td>(k_{eo}) (/min)</td>
<td>0.242</td>
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</tbody>
</table>

A PE is the difference between a measured blood concentration \((C_m)\) and the model predicted concentration \((C_p)\) in terms of \(C_p\). PE can be expressed as a percentage, defined as:

\[
PE = \frac{C_m - C_p}{C_p}
\]

Using this definition, the PEs for all the population models tested were computed at every measured data point. The overall inaccuracy of the model was determined by computing the MDAPE, defined as:

\[
MDAPE = \text{median}\{|PE_1|, |PE_2|, ..., |PE_n|\}
\]

where \(n\) is the total number of samples in the study population. The MDPE, a measure of model bias, was also computed for each model.

\(C_{E50}\) = 50% effective concentration; \(CV = \text{coefficient of variation (omega from NONMEM)}\); \(E_{\max}\) = maximal effect; \(E_0\) = predrug effect; \(\gamma\) = the steepness of the concentration-effect relationship; \(k_{eo}\) = first-order rate constant characterizing effect site equilibration kinetics; MDAPE = median absolute prediction error; MDAPE = median prediction error; 95% CI = computed from a bootstrap technique with 1,000 replications.

AZD-3043 is a potent GABA\(_A\)-mediated chloride channel opener in the blood. A more complete description of the metabolic profile of AZD-3043 was observed, with the formation of the corresponding carboxylic acid metabolite, THRX-108893, being evident in whole blood. Upon metabolism of AZD-3043, the formation of the corresponding carboxylic acid metabolite, THR\(_{108893}\), was observed, suggesting that AZD-3043 is metabolized by esterase present in the blood. A more complete description of the in vitro and in vivo pharmacokinetic profile of AZD-3043 will be the subject of a future publication.
AZD-3043, but not its carboxylate metabolite, THRX-108893, inhibited GABA\(_A\) ([\(^{15}\)S]t-butyl bicyclophosphorothionate) binding in the *in vitro* radioligand binding studies. AZD-3043 had little or no affinity for a variety of other CNS-expressed receptors and ion channels. The binding and electrophysiological data are consistent with the proposal that AZD-3043 is a positive allosteric modulator of GABA\(_A\) activity and, as such, should potentiate GABA\(_A\)-mediated inhibitory neurotransmission in the CNS.

AZD-3043 produced hypnosis in rats with rapid onset after bolus IV administration. Despite similar potencies in experiments with the embryonic rat cortical neurons, AZD-3043 was approximately 2.5-fold more potent than propandid after bolus IV dosing. Importantly, THRX-108893 and 1-propanol, the primary metabolites of AZD-3043, were either inactive or only very weak hypnotic agents. Upon return of the righting reflex, the time to behavioral recovery was rapid and remained so with increasing doses of AZD-3043 or propandid, in contrast to the findings with propofol. After bolus IV administration to rats, AZD-3043 produced a transient and dose-dependent suppression of electroencephalographic activity with rapid onset of action. Such activity is consistent with a sedative–hypnotic agent possessing rapid CNS penetration and clearance. The recovery of the electroencephalogram after suppression with AZD-3043 was more rapid and less influenced by increasing bolus dosage than was seen with propofol. In general, the electroencephalographic (BIS) data obtained in the porcine model paralleled the findings from the rodent studies, although a clear limitation of the pig experiments was the concomitant administration of isoflurane (for ethical reasons).

In the majority of general anesthetic procedures, opioids are coadministered with hypnotic agents. Synergy between a hypnotic agent and an opioid is commonly observed, the analgesia conferred by the opioids allowing the dose of hypnotic to be reduced. In this study, the leftward shift in the hypnosis dose response curve of AZD-3043 was greater than that of propofol when remifentanil was coadministered but similar upon fentanyl coadministration. Upon bolus dosing of remifentanil and AZD-3043, esterases may have been saturated transiently, and the synergy with these agents was thus more marked than the remifentanil-propofol combination. The similarly large reduction in the hypnotic dose of propandid, upon coadministration of remifentanil, supports such an hypothesis. The data are consistent with a reduction of the required clinical dose of AZD-3043 when used in combination with opioids.

The most important finding in this study was that the time to emergence from AZD-3043–induced hypnosis was rapid and relatively unaffected by the duration of infusion, in contrast to the findings with propofol. It was also apparent that the infused dose of AZD-3043 required less alteration, in comparison with that of propofol, to maintain a consistent depth of hypnosis throughout (as monitored by the admittedly subjective observation of noxious stimulus-induced paw withdrawal). The infusion data for AZD-3043 are consistent with a short, and constant, 80 or 50% decrement time. These results suggest that the use of AZD-3043 in the clinic may allow more rapid and predictable patient emergence, more precise control of hypnotic depth, and less requirement for dose titration upon prolonged infusion than does propofol. Of course it is important to emphasize that the pharmacokinetic simulations from the animal models may not be predictive of human pharmacology and require confirmation in man.

From a pharmaceutics perspective, AZD-3043 is a “soft drug.” Soft drugs are molecules that are purposefully designed to be rapidly metabolized (metabolically labile), in anesthesia, the soft drug concept is useful because it enables precise titration to effect and rapid recovery. The short-acting opioid remifentanil and \(\beta\)-blocker esmolol are familiar applications of this pharmacaceutic approach in perioperative practice. AZD-3043, CNS7056, and MOC-etomidate (and others) are all more recent examples of the soft drug trend in anesthesia drug discovery. The first-in-man studies of AZD-3043 have now been completed, and the data analysis is ongoing. Given the unique requirements of anesthesia therapeutics, we can expect to see more soft drugs in anesthesiology in the future.

References


10. Ko JC, Williams BL, Smith VL, McGrath CJ, Jacobson JD: Comparison of Telazol, Telazol-ketamine, Telazol-xylazine, and Telazol-ketamine-xylazine as chemical restraint and an-


