METABOLISM AND DISPOSITION OF A POTENT GROUP II METABOTROPIC GLUTAMATE RECEPTOR AGONIST, IN RATS, DOGS, AND MONKEYS

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Received April 4, 2005; accepted June 15, 2005

ABSTRACT:

Metabolism and disposition of MGS0028 [(1R,2S,5S,6S)-2-amino-6-fluoro-4-oxobicyclo[3.1.0]hexane-2,6-dicarboxylic acid monohydrate], a potent group II metabotropic glutamate receptor agonist, were examined in three preclinical species (Sprague-Dawley rats, beagle dogs, and rhesus monkeys). In rats, MGS0028 was widely distributed and primarily excreted in urine as parent and as a single reductive metabolite, identified as the 4R-isomer MGS0034 [(1R,2S,4R,5S,6S)-2-amino-6-fluoro-4-hydroxybicyclo[3.1.0]hexane-2,6-dicarboxylic acid]. MGS0028 had a low brain to plasma ratio at efficacious doses in rats and was eliminated more slowly in rat brain than in plasma. Exposure increased proportionally (1–10 mg/kg p.o.) in rats, with bioavailability >60% at all doses. However, bioavailability was only ~20% in monkeys, and MGS0034 was found in relatively high abundance in plasma. In dogs, oral bioavailability was >60%, and the metabolite was not detected. In vitro metabolism was examined in liver subcellular fractions (microsomes and cytosol) from rat, dog, monkey, and human. Reductive metabolism was observed in rat, monkey, and human liver cytosol incubations, but not in dog liver cytosol incubations. No metabolism of MGS0028 was detected in incubations with liver microsomes from any species. Similar to in vivo results, MGS0028 was reduced in cytosol stereospecifically to MGS0034. The rank order of in vitro metabolite formation (monkey > rat > human > dog) was in agreement with in vivo observations in rats, dogs, and monkeys. Based on the observation of species difference in reductive metabolism, rat and monkey were recommended to be the preclinical species for further characterization prior to testing in humans. Finally, allometric scaling predicts that human pharmacokinetic parameters would be acceptable for further development.

Metabotropic glutamate receptors (mGluRs) have received significant attention as therapeutic targets for the treatment of CNS disorders (Danyasz et al., 1995; Helton et al., 1998; Cartmell et al., 1999; Johnson et al., 2002, Takamori et al., 2003). Unlike ionotropic glutamate receptors, which are ion channels, mGluRs are members of the G-protein-coupled receptor superfamily and modulate synaptic transmission through second messenger systems that can both stimulate and inhibit neurons. The mGluR family of receptors is cataloged into three groups, consisting of eight subtypes (group I, mGluR1 and mGluR5; group II, mGluR2 and mGluR3; and group III, mGluR4, mGluR6, mGluR7, and mGluR8) (Nakanishi, 1992; Nakanishi and Masu, 1994; Pin and Duvoisin, 1995; Conn and Pin, 1997; Schoepp et al., 1999) and are located both pre- and postsynaptically.

Glutamate, the major excitatory amino acid in the CNS, binds mGluRs extracellularly, thereby initiating intracellular cascade events. Whereas group I mGluRs are found primarily postsynaptically and ultimately increase neuronal excitability (Conn and Pin, 1997; Pin et al., 1999, Milovanovic et al., 2002), group II and III mGluRs are primarily localized presynaptically and are coupled to the inhibitory G-proteins Gi and G0, which reduce cellular levels of the second messenger cAMP and inhibit glutamate release (Flor et al., 1995; Emile et al., 1996; Makoff et al., 1996; Conn and Pin, 1997; Pin et al., 1999; Kammermeier et al., 2003). Group II metabotropic glutamate receptors (mGluR2 and mGluR3), although not completely characterized, are thought to modulate neuronal excitability by regulating synaptic glutamate levels. Thus, agonists for these receptors are implicated in therapeutic indications including schizophrenia, anxiety, and addiction (Helton et al., 1997; Moun et al., 1997; Helton et al., 1998; Moghadam and Adams, 1998; Swanson and Schoepp, 2002) and have even advanced to the level of human clinical trials (Levine et al., 2003; Schoepp et al., 2003).

ABBREVIATIONS: mGluR, metabotropic glutamate receptor; HmGluR, human mGluR; CNS, central nervous system; CSF, cerebral spinal fluid; HPLC, high performance liquid chromatography; LC-MS/MS, liquid chromatography-tandem mass spectrometry; PFB, pentafluorobenzyl bromide; PK, pharmacokinetic; MGS0028, (1R,2S,5S,6S)-2-amino-6-fluoro-4-oxobicyclo[3.1.0]hexane-2,6-dicarboxylic acid monohydrate; MGS0034, (1R,2S,4R,5S,6S)-2-amino-6-fluoro-4-hydroxybicyclo[3.1.0]hexane-2,6-dicarboxylic acid; MGS0025, [(1R,2S,4S,5S,6S)-2-amino-6-fluoro-4-hydroxybicyclo[3.1.0]hexane-2,6-dicarboxylic acid; MGS0026, (--)2-oxa-4-aminobicyclo hexane-4,6-dicarboxylic acid; SAX, strong-anion exchange; AUC, area under the curve; AUCN, dose-normalized AUC; AUMC, area under the moment curve; CLp, plasma clearance; amu, atomic mass unit(s); LY379268, (--)2-aminobicyclo[3.1.0]hexane-2,6-dicarboxylic acid; ADME, absorption, distribution, metabolism, and excretion; MLP, maximum lifespan potential.
MGS0028, the mGluR2/3 agonist, is a constrained glutamic acid analog (Fig. 1) with in vitro potency at HmGluR2 and HmGluR3 of 0.57 and 2.07 nM, respectively (Nakazato et al., 2000). In vivo, MGS0028 inhibits phencyclidine-induced head-weaving behavior and hyperactivity in the rat at oral doses of 0.3 to 3 mg/kg (Nakazato et al., 2000) and is active in the conditioned avoidance response model in rats over the same dose range (Takamori et al., 2003). The desirable in vitro potency and in vivo efficacy profiles make this compound an attractive candidate for clinical development. Thus, to further characterize suitability and to select the appropriate preclinical species for future development, the metabolic and disposition profile in Sprague-Dawley rats, beagle dogs, and rhesus monkeys was characterized. Human pharmacokinetic (PK) parameters were predicted with allometric scaling methods. In addition, since brain penetration is required for an mGluR2/3 agonist to reach the intended target, analysis of brain penetration and brain levels over time in rats was carried out.

Materials and Methods

Materials. MGS0028, MGS0034 ([1R,25,4R,5,5S,6S]-2-amino-6-fluoro-4-hydroxybicyclo[3.1.0]hexane-2,6-dicarboxylic acid, the reductive metabolite of MGS0028), and MGS0025 (the 4S diastereomer of MGS0034) were synthesized at Taisho Pharmaceuticals Co. (Nakazato et al., 2000). [14C]MGS0028 was synthesized by Amersham Biosciences Inc. (Piscataway, NJ) (specific activity 907.13 KBq/mg and radiochemical purity greater than 97% by thin-layer chromatography). The radioactive material was diluted with unlabeled compound synthesized by Taisho Pharmaceutical Co. when necessary. The chemical structures of the compounds and the 14C-labeled position on MGS0028 (indicated by an asterisk) are shown in Fig. 1. Blank plasma was received from the following sources: rat plasma in EDTA, Dynal Biotech (Lake Success, NY); dog plasma in EDTA, Merck Research Laboratories-Rahway Beagle Facility; and rhesus monkey plasma in EDTA, Biochem Pharmaceutica (Los Angeles, CA). All solvents were purchased from Fisher Scientific Co. (Pittsburgh, PA) in HPLC grade. The compounds are monohydrates and all data calculations were performed using the free-base molecular weight.

In Vitro Metabolism. Rat and human liver microsomes were purchased from In Vitro Technologies (Baltimore, MD), and human liver microsomes were pooled from 10 individuals. Dog and rhesus monkey liver microsomes were obtained from Merck Research Laboratories, Rahway, and liver cytosol were obtained from Merck Research Laboratories, West Point. Liver cytosol (1 mg/ml protein concentration) from rat, human, monkey and dog were incubated with 1 or 10 μM MGS0028 in the presence or absence of 1 mM NADPH (100 mM Tris buffer, pH 7.4). The reactions were terminated after 1 h of incubation at 37°C with the addition of 2 volumes of acetonitrile. Prior to LC-MS/MS analyses, the incubation samples were processed through solid phase extraction with Isolute SAX (strong-anion exchange) SPE columns (2 g/15 ml). MGS0028 and the metabolite MGS0034 were separated on a YMC ODS-4Q HPLC column (3 μm, 4.6 × 150 mm; YMC, Inc., Wilmington, NC) with an isocratic mobile phase (95% water and 5% acetonitrile containing 0.1% acetic acid) at a flow rate of 0.5 ml/min. Tandem mass spectrometric (MS/MS) detection was carried out using electrospray (Micromass Quattro Ultima; Waters, Milford, MA) in negative ion mode for the parent compound MGS0028 (m/z 216 → 108), the metabolite MGS0034 (m/z 218 → 154), and MGS0025, which is the 4S diastereomer of the metabolite.

Animal Experiments. All animal experiments conducted at Merck Research Laboratories followed procedures set forth by the Merck Research Laboratories Institutional Animal Care and Use Committee, and all guidelines were in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Experiments performed at Taisho Pharmaceutical Co. followed similar internal guidelines.

ADME Studies: Absorption, Tissue Distribution, Metabolism, and Excretion Using Radiolabel and Radioluminography in Rats. Animals. Male Sprague-Dawley rats (8–9 weeks old) were obtained from Charles River (Yokohama, Japan). All animals were acclimated for at least 1 week before entering a study. The animals were provided with water and standard laboratory diet (MF; Oriental Yeast Co., Tokyo, Japan) ad libitum during acclimation, and were fasted overnight (about 18 h) before and 6 h after dosing, except in the studies to examine the dietary effect and distribution of radioactivity after administration.

Dosing Solutions. [14C]MGS0028 was dissolved in isotonic saline containing 0.3% Tween 80 for both intravenous (i.v.) and oral (p.o.) administration. The radiolabel doses and dosing volumes were 3.7 MBq/ml/kg for i.v. administration and 1.8 to 3.7 MBq/5 ml/kg for p.o. administration. In the radioluminography studies, the radiolabel dosing volume was 7.4 MBq/5 ml/kg.

Blood Concentrations of Radioactivity. Blood samples were taken from the tail vein at 2, 10, and 30 min or 1, 3, 6, 8, and 24 h after i.v. administration of [14C]MGS0028, and at 10, 20 and 30 min or 1, 2, 4, 6, 8, and 24 h after p.o. administration. Each blood sample was solubilized with a mixture of 1 ml of Soluene-350 (PerkinElmer Life and Analytical Sciences, Boston, MA) and 0.5 ml of isopropanol, and decolorized with addition of 30% H2O2. Then, 10 ml of liquid scintillation cocktail (Hionic Fluor, PerkinElmer Life and Analytical Sciences) was added and the radioactivity was counted.

Radioluminography. After p.o. administration of [14C]MGS0028, rats were sacrificed by ether anesthesia at 1, 4, 8, and 24 h after dosing. The carcass was frozen in a dry ice-ice hexane mixture at approximately −80°C, embedded in 5% sodium carboxymethyl cellulose on a microtome stage, and refrozen. Forty-micrometer thick sections were prepared by a Cryomacrotom (PMV450MP, LBK; Leica, Wetzlar, Germany) and freeze-dried at −20°C. The sections were x-rayed in a protection membrane, placed in contact with an imaging plate (BAS-III; Fuji Photo Film, Japan, Tokyo, Japan), exposed under room temperature for 16 h, and developed to prepare radioluminograms using a Bioimage analyzer (Model BAS2000; Fuji Photo Film, Japan). The section taken at 4 h was also stained with hematoxylin-eosin.

Brain and Cerebrospinal Fluid (CSF) Concentrations of Radioactivity. After i.v. administration of [14C]MGS0028, rats were anesthetized with 125 mg/kg ketamine (Ketaral 50; Sankyo Co., Tokyo, Japan), and CSF was collected from the cisterna magna at 1, 2, 4, and 6 h; each aliquot was mixed with liquid scintillator, and radioactivity was counted. Blood was then collected from the abdominal aorta and the animals were decapitated immediately. Plasma was obtained by centrifugation of blood; then, liquid scintillator was added and the radioactivity counted. Half of the cerebrum and cerebellum was excised and weighed. The choroid plexus was removed from the other side of the cerebrum and was put into scintillation counting vials for solubilization, and the radioactivity was counted. Brain tissues were homogenized in 4
lit.

Rat urine (0.1 ml, 0–8 h post 1 mg/kg p.o. dose) was mixed with 1 ml of water (ratio 2:98 containing 5% trifluoroacetic acid) at a flow rate of 0.8 ml/min. Radioactivity was detected by a radioactivity monitor (Ramon93; Raytest, Straubenhardt, Germany).

**Liquid Chromatographic Mass Spectrometric Analysis of Urinary Metabolite:** Rat urine (0.1 ml, 0–8 h post 1 mg/kg p.o. dose) was mixed with 1 ml of methanol containing 1% trifluoroacetic acid and centrifuged at 11,000 g for 10 min. The supernatant was dried under a gentle stream of nitrogen, and the residue was dissolved in 0.1 ml of acetonitrile and subjected to derivatization.

The PFB derivatives of MGS0034 and its metabolite(s) were prepared by addition of pentafluorobenzyl bromide (0.1 ml) and disopropylethylamine (0.1 ml) followed by vigorous mixing and heating at 70°C for 2 h. The derivatization was terminated by evaporation of reagents under a gentle stream of nitrogen. The residue was dissolved in 0.2 ml acetonitrile, and an aliquot (30 μl) was injected onto a HPLC system (LC10A; Shimadzu, Kyoto, Japan) with a reverse-phase column (Capcel-pod ODS, 5 μm, 4.6 × 150 mm; Shiseido, Tokyo, Japan). The analyte was eluted with a linear gradient (38% to 90% acetonitrile over 30 min) at a flow rate of 1.0 ml/min; the eluent was collected in 1.0-ml fractions, and each fraction was measured for radioactivity. The fraction corresponding to the peaks of PFB derivatives of [14C]MGS0028 and the metabolite were analyzed by a LC-MS/MS system (API 3000 mass spectrometer; PerkinElmerSciex Instruments, Boston, MA).

**Urine and Fecal Excretion of Radioactivity:** Fasted rats were individually housed in glass metabolic cages after i.v. or p.o. administration of [14C]MGS0028. Urine was collected separately over the periods of 0 to 8, 8 to 24, 24 to 48, and 48 to 72 h and feces were collected in 24-h intervals until 72 h. After collection, each fraction was weighed. An aliquot of each urine sample was mixed with liquid scintillator and the radioactivity was counted. The feces were homogenized and solubilized with Soluene-350 and the radioactivity was counted.

**Measurement of Radioactivity:** Radioactivity in each test sample was measured by a liquid scintillation counter (LS6000TA; Beckman Coulter, Fullerton, CA). Quenching was corrected by means of the external standard method. Concentrations of radioactivity were expressed as concentration equivalents based on the specific activity in the dosing solution.

**Pharmacokinetic Studies in Rats, Dogs, and Monkeys.** Animals. Male Sprague-Dawley rats (Harlan, Indianapolis, IN) were habituated to the vivarium for at least 3 days before entering a study. Jugular vein cannulae were implanted for serial bleeding, and the animals were allowed to recover for an additional 3 days. MGS0028 was administered i.v. at 3 and 10 mg/kg (0.3% Tween 80 in saline, 0.5 ml/kg, n = 3) and p.o. at doses of 1, 3, and 10 mg/kg (0.3% Tween 80 in water, 2 ml/kg, n = 3). Blood samples were collected at 5 (i.v. only), 15, and 30 min, and 1, 2, 4, 6, 8, and 24 h postdose.

Male rhesus monkeys weighing 8 to 10 kg were fasted for 18 h before dosing and 8 h thereafter. The animals received an i.v. dose of MGS0028 at 1 mg/kg (saline, 0.1 ml/kg, n = 3) and a p.o. dose at 5 mg/kg (water, 5 ml/kg, n = 3). Blood samples were collected at 0, 5 (i.v. only), 15, and 30 min, and 1, 2, 4, 6, 8, and 24 h postdose. Male beagle dogs weighing 10 to 12 kg were fasted for 18 h before dosing and 4 h thereafter. The animals received an i.v. dose of MGS0028 at 0.3 mg/kg (saline, 0.1 ml/kg, n = 3). Prior to the oral study, the dogs were pretreated with MGS0028 at 0.1 mg/kg/day p.o. for 3 days to allow the animals to develop tolerance to the potential emetic effect of MGS0028. Before the study day, the animals were fasted overnight and received a dose of MGS0028 at 0.1 or 0.3 mg/kg p.o. (water, 5 ml/kg, n = 3). Blood samples were collected at 0, 5 (i.v. only), 15, and 30 min, and 1, 2, 4, 6, 8, and 24 h postdose. In all cases, the plasma samples were obtained upon centrifugation of blood samples and stored at −20°C until analyzed.

**LC-MS/MS Assay.** A LC-MS/MS method was developed for the simultaneous analysis of nonderivatized MGS0028 and its metabolite MGS0034. Plasma samples (50 μl) calibration, and quality control standards underwent a protein precipitation procedure on a Tomtec robotic system (Tomtec, Orange, CT) in a 96-well plate format. Calibration curves for both MGS0028 and MGS0034 were prepared in species-matched blank plasma with a typical dynamic range of 2 to 2000 ng/ml (higher if required). In cases where the metabolite standard was unavailable, the stereoisomer, MGS0025, was used as a surrogate. LY 379268 was used as the analytical internal standard, which was spiked in the precipitation solvent. After removal of the protein pellets, the samples were processed in one of two ways: method 1, in which the samples were diluted with 2 volumes of 0.05% formic acid and 100 μl of the final mixture injected onto the LC/MS/MS system, or method 2, in which the samples were further purified by solid phase extraction using SAX cartridges (96-well plate format, 25-μg capacity Isolute SAX cartridges, obtained from Jones Chromatography, Lakewood, CO). Washing steps included methanol, water, acetonitrile/water (9:1) at 1 μl each, followed by elution with 400 μl of acetonitrile/water (9:1) containing 1% trifluoroacetic acid. The final eluent was either injected directly or after drying and reconstitution in 100 μl of 0.05% formic acid (5 μl).

Samples prepared by method 1 were analyzed by liquid chromatography with mass detection on a 50 × 4.6 mm Phenomenex AQUA column (polar end-capped C18; Phenomenex, Torrance, CA) using isocratic elution at 95% water containing 0.05% formic acid and 5% acetonitrile at 0.8 ml/min. The eluent was split 4:1 such that <0.2 ml/min was flowing into the mass spectrometer. For samples prepared using SAX after protein precipitation (method 2), chromatographic separation was achieved on a 150 × 2 mm YMC Hydrosphere column (polar end-capped C18) with the same isocratic mobile phase elution at a flow rate of 0.2 ml/min. MS/MS detection was carried out using electrospray (Micromass Quattro Ultima; Waters) in negative ion mode for the parent compound MGS0028 (m/z 216 → 108), the metabolite MGS0034 (m/z 218 → 154), and internal standard (m/z 186 → 98.5). The lower limit of quantitation ranged from 4 to 32 ng/ml (0.02–0.15 μM), and was assay-dependent.

**Calculation of Pharmacokinetic Parameters.** Pharmacokinetic parameters were calculated using a template developed in-house. Calculations are based on the noncompartamental model using the linear trapezoidal estimation method.

The dose-normalized AUC (μM · h) is defined as AUC = AUC/Dose, and the area under the moment curve (μM · h) for I.V. experiments is calculated as AUMC = T(1)2 · C(1)/2 + Σ T(n)(T(n) − T(n − 1)) · (C(n) − C(n − 1))/2 − C(N) · (T(N)/N)2 + C(N)/N2, where the last two terms are omitted when the extrapolation terms in AUC are omitted. The concentration at time 0, C0 (μM), will be 0 after a compound is dosed orally or by infusion.

**Plasma Protein Binding and Blood/Plasma Partition.** Plasma from rat, dog, monkey, and human was spiked with [14C]MGS0028 at concentrations of 1 and 10 μM. Aliquots (200 μl, n = 3 per concentration) were transferred to ultracentrifuge tubes and centrifuged for 60 min at 1800g at 37°C. An aliquot of the supernatant (50 μl) from each tube was mixed with 10 ml of Pico-Fluor 40 (PerkinElmer Life and Analytical Sciences) and counted with a liquid scintillation counter.

Fresh blood from male Sprague-Dawley rats and humans was spiked with [14C]MGS0028 at a concentration of 1 μM. These samples were incubated at 37°C. A preliminary study established that 5 min was sufficient to reach equilibrium. Aliquots were taken and centrifuged to separate the plasma from red blood cells. Plasma (100 μl) was dissolved in scintillant and counted with a Beckman Coulter scintillation counter LS6500.

**Allometric Scaling of Pharmacokinetics Parameters.** The effective human half-life (t1/2) included in Table 1, since this value was consistent for all preclinical species tested. The human PK parameters for plasma clearance (CLp) and volume of distribution (Vd) were calculated using protocols described by Obach et al. (1997). Methods used for Vd included Vd1, Vd2, and Vd3b. For CLp, methods C3b, C3c, and C3d were used, as well as the average predicted human Vd (0.099 l/kg) using the formula CLp = (0.693 · V1/2)/t1/2.

**Results**

Absorption. MGS0028 was found to be well absorbed in rats following p.o. dosing of [14C]MGS0028. Pharmacokinetic parameters derived from the total radioactivity are summarized in Table 1. Upon an i.v. dose of 1 mg/kg [14C]MGS0028, total radioactivity reached 5.91 micromolar Eq at the first sampling point of 2 min and declined biexponentially thereafter, with a half-life (t1/2) of 2.4 h. To assess the
impact of food, [14C]MGS0028 was administered orally to both fasted and fed rats. Following a p.o. dose of 1 mg/kg [14C]MGS0028 to fasted rats, total radioactivity peaked at 1.15 micromol Eq at 0.8 h postdose. In fed animals, the absorption process was noticeably slower, peaking at a lower level and at a later time (0.49 micromol postdose). Nonetheless, the AUC0–24 h values were similar in fasted and fed animals, (3.40 and 3.63 Eq at 1.7 h). Nonetheless, the AUC0–24 h values were similar in fasted and fed animals (3.40 and 3.63 μM h), and p.o. administration. The radiolabel doses and dosing volumes were 3.7 MBq/ml/kg for fasted rats, total radioactivity peaked at 1.15 micromolar Eq at 0.8 h postdose. In fed animals, the absorption process was noticeably slower, peaking at a lower level and at a later time (0.49 micromolar Eq at 1.7 h). Nonetheless, the AUC0–24 h values were similar in fasted and fed animals (3.40 and 3.63 μM h) and absorption was good with about 60% bioavailability.

**Tissue Distribution.** Distribution of radioactivity to tissues following a dose of 1 mg/kg p.o. [14C]MGS0028 was examined by radioluminography. At 1 h postdose, relative levels of radioactivity were ranked in four groups: kidney, bladder urine, intestinal contents, thymus, liver, and harderian gland, blood, lung, and skin, heart, testis, epididymis, pancreas, submandibular gland, adrenal gland, spleen, and small intestine > > cerebrum and cerebellum (undetectable). Although most parts of the brain contained an undetectable level of radioactivity, the area postrema showed a relatively high level of radioactivity (Fig. 2). The localization of radioactivity was confirmed with hematoxylin-eosin staining of the slice. At 4 h postdose, radioactivity was detected in kidney, bladder urine, intestinal contents, thymus, liver, pancreas, and harderian gland, which all appeared to be higher than that in the blood. At 8 h postdose, radioactivity was detected in all the listed tissues with the exception of the pancreas. At 24 h postdose, radioactivity could still be detected in the kidney, intestinal contents, thymus, liver, and the hardierian gland.

To further characterize CNS penetration, the distribution of radioactivity in the brain, choroid plexus, and CSF relative to blood and plasma was studied in rats following a dose at 1 mg/kg i.v. [14C]MGS0028. As shown in Fig. 3, levels of radioactivity in the plasma, cerebrum, and CSF reached 0.87, 0.06, and 0.02 micromolar Eq at 1 h postdose, respectively. The brain to plasma ratio of radioactivity was 0.07 at 1 h, corresponding to the maximum plasma concentration ($C_{max}$), and 0.39 at 6 h. The elimination of the radioactivity in the brain was slower than that in the plasma and CSF. No radioactivity was detected in the choroid plexus.

**Metabolism.** In vivo metabolism of [14C]MGS0028 was studied in rats following a dose at 1 mg/kg p.o. in fasted rats. Figure 4 shows the radiochromatograms of rat urine samples, which demonstrated the presence of a single major metabolite. The metabolite consisted of 90% of the total radioactivity in 0– to 8-h urine samples and was the only peak detected in 8- to 24-h urine samples. Identification of the metabolite was carried out by derivatization with PFB, followed by liquid chromatography/mass spectrometry with atmospheric pressure chemical ionization detection. The [MH]$^+$ of the derivatized metabolite (M1–3PFB) was at $m/z$ 760, which was 2 amu higher than the parent drug (MGS0028–3PFB) at $m/z$ 758, indicating that the metabolite contained an alcohol group as a result of metabolic reduction of the ketone. Subsequently, two diastereoisomers of the alcohol were synthesized: MGS0025 (4S-isomer) and MGS0034 (4R-isomer) (Nakazato et al., 2000). After derivatization with PFB and HPLC separation, retention time comparison to authentic standard demonstrated that MGS0028 was stereospecifically metabolized in rats to form the 4R-isomer (MGS0034). MGS0034 was also identified in monkey plasma, whereas it was not detected in dog plasma or urine.

For species comparison, the in vitro metabolism of MGS0028 was investigated with rat, monkey (rhesus), dog and human liver subcellular fractions (microsomes and cytosol). MGS0028 was stable in hepatic microsomal incubations across the four species and the reductive metabolite MGS0034 was not detected in liver microsomal incubations (data not shown). However, the metabolite was stereospecifically formed in rat, monkey, and human liver cytosols, which suggested the reductive metabolism of MGS0028 was mediated by cytosolic carbonyl reductase. Interestingly, the metabolite was not formed in dog cytosol. The rate of the formation was quantitatively assessed at a pharmacologically relevant concentration (1 μM) (Fig.

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<tr>
<td>$C_{max}$ (μM)</td>
<td>5.91 ± 1.84</td>
<td>1.15 ± 0.26</td>
</tr>
<tr>
<td>$t_{max}$ (h)</td>
<td>–</td>
<td>0.8 ± 0.2</td>
</tr>
<tr>
<td>AUC0–24 h (μM h)</td>
<td>5.94 ± 1.26</td>
<td>3.40 ± 0.23</td>
</tr>
<tr>
<td>$F$ (%)</td>
<td>57</td>
<td>61</td>
</tr>
<tr>
<td>$t_{1/2}$ (h)</td>
<td>2.4 ± 0.6</td>
<td>2.2 ± 0.2</td>
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</table>

Area postrema.

FIG. 2. Radioluminogram of a rat brain 4 h after oral administration of [14C]MGS0028 at 1 mg/kg. The radiolabel dosing volume was 7.4 MBq/5 ml/kg. Radioactivity was only detected in the area postrema. The localization of radioactivity was confirmed with hematoxylin-eosin staining of the slice.

FIG. 3. Radioactivity in rat blood, plasma, brain, and CSF following i.v. administration of [14C]MGS0028 (3.7 MBq/ml/kg). Each point represents the mean ± S.D. of three animals.

### Table 1

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only 4.7% eliminated in feces. A similar excretion pattern was observed after p.o. dosing, i.e., 93% in urine and 3.9% in feces (Table 2). Comparable urinary excretion after i.v. and p.o. administration suggests total radioactivity was primarily eliminated through renal clearance.

**Pharmacokinetics in Rats, Monkeys, and Dogs.** For all species, plasma levels were quantitatively measured for both MGS0028 and the reductive metabolite MGS0034. The PK parameters in rats, as well as dogs and monkeys, are provided in Table 3, and the rat plasma concentration-time curves at 3 mg/kg are shown in Fig. 7. In rats, following administration of MGS0028 at 3 mg/kg i.v., mean $C_{Lp}$ is moderate at 16.2 ml/min/kg and the corresponding volume of distribution ($V_d$) value is 0.5 l/kg, which is nearly on the order of total body water. Oral bioavailability and dose proportionality studies in rats were conducted at 1, 3, and 10 mg/kg. The corresponding mean $C_{max}$ values were 1.0, 2.7, and 6.0 $\mu$M, and exposure, as measured by $AUC_{0-24\,h}$ increased proportionally with the increase in dose. At all doses, oral bioavailability was greater than 60%. In both i.v. and p.o. dose studies, the reductive metabolite MGS0034 was detected, and the metabolite to parent drug AUC ratio was variable, ranging from 0.3 to 0.8.

The PK parameters measured in rhesus monkeys are provided in Table 3, and the plasma concentration-time curves are shown in Fig. 8. Following administration of 1 mg/kg i.v. MGS0028, the $C_{Lp}$ was modest at 8.0 ml/min/kg and the mean $V_d$ was at 0.6 l/kg (nearly the volume of total body water). Again, an effective $t_{1/2}$ of 1 h was observed. A study was conducted at 5 mg/kg p.o. in solution (water), resulting in a mean $C_{max}$ of 1.0 $\mu$M. Following p.o. dosing, plasma levels of MGS0028 are sustained for at least 8 h, presumably due to slow absorption, yet bioavailability was relatively modest, at only about 20%. The reductive metabolite MGS0034 was found in relatively high abundance in plasma, reaching levels comparable to or exceeding the parent drug beyond 4 h postdose. The AUC ratio between the metabolite and the parent drug was 0.3 and 0.9 following i.v. and p.o. administration, respectively.

The dog PK parameters and plasma concentration-time curves are shown in Table 3 and Fig. 9. Following administration of MGS0028 at 0.3 mg/kg i.v., $C_{Lp}$ and $V_d$ were low at 2.8 ml/min/kg and 0.1 l/kg, respectively, and the effective $t_{1/2}$ was consistently short at 1 h. Oral studies were conducted at two doses, 0.1 and 0.3 mg/kg, both given in solution using 0.3% Tween 80 in water, after 3 days of administration at 0.1 mg/kg p.o., to minimize compound-induced emesis. Plasma taken prior to compound administration on the study day showed that there was no measurable compound present from predosing. Mean $C_{max}$ values increased nearly proportionally from 0.1 mg/kg to 0.3 mg/kg, at 1.5 $\mu$M and 4.0 $\mu$M. Oral bioavailability was about 60% to 70%. In dogs, the reductive metabolite MGS0034 was not detected in plasma following either i.v. or p.o. administration. Furthermore, urine samples collected after i.v. administration also lacked the metabolite.

**Plasma Protein Binding and Blood/Plasma Partition.** The mean plasma protein binding values in three animal species and humans are provided in Table 4. At a pharmacologically relevant concentration (1 $\mu$M), the mean unbound fraction in rat, dog, monkey (rhesus), and human plasma was near 70% (similar values were obtained at 10 $\mu$M). Thus, the plasma protein binding of MGS0028 is very low in all four species. The blood to plasma partition of MGS0028 was 0.76 ± 0.02 and 0.64 ± 0.02 in rats and humans, respectively. A time course study indicated that the equilibrium between plasma and red blood cells was reached by 5 min at 37°C. The blood to plasma ratio (slightly above 0.5) suggests that MGS0028 is primarily partitioned into plasma rather than red blood cells.

**Allometric Scaling of Pharmacokinetic Parameters.** The human PK parameters for $C_{Lp}$ and $V_d$ were predicted using allometric scaling of the PK data from the preclinical species examined. The measurable
effective $t_{1/2}$ in all three preclinical species was about 1 h; thus, $t_{1/2}$ in human was assumed to be the same for the purpose of scaling. The other PK parameters were fitted using a number of methods to predict ranges (Obach et al., 1997). All methods resulted in fairly narrow ranges, which are presented in Table 5. Human $CL_{p}$ is predicted to be modest, at between 1 and 4 ml/min/kg (Fig. 10). Volume of distribution is also predicted to be low, at 0.08 to 0.11 l/kg, indicating a preference for the blood compartment.
Discussion

MGS0028 is a group II metabotropic glutamate receptor agonist with improved potency (EC$_{50}$ at HmGluR2 and HmGluR3 is 0.57 nM and 2.07 nM, respectively) over current compounds in its class, including the leading clinical candidate, LY354740 (EC$_{50}$ 18.3 nM and 62.8 nM in the same assay) (Nakazato et al., 2000; Johnson et al., 2002). LY354740 established proof of concept for anxiety in humans for mGluR2/3 agonists, showing efficacy in the CO$_2$-induced anxiety model and fear-potentiated startle response in humans (Grillon et al., 2003; Levine et al., 2003). At present, MGS0028 has demonstrated efficacy in animal models of schizophrenia and anxiety, including the phencyclidine-induced hyperactivity and fear-avoidance models in rats (Nakazato et al., 2000; Takamori et al., 2003). Having established desirable in vitro potency and in vivo efficacy, further analysis of the metabolism and disposition of MGS0028 was done to determine whether MGS0028 would be an attractive candidate for clinical development. Thus, rat ADME and preclinical multispecies PK was examined and scaled using allometric methods to predict key human PK parameters.

Since mGluR2/3 receptors are located in the brain, special emphasis was given to brain penetration and localization when analyzing total body distribution. Rat ADME and multispecies PK analysis of MGS0028 indicate that this compound is well absorbed in all preclinical species and is widely distributed. However, for a CNS drug, MGS0028 is poorly brain-penetrant. This is probably due to the polarity of MGS0028, since it was found to not be a P-glycoprotein substrate and suffers from poor permeability (data not shown). MGS0028 is highly water-soluble, and distribution studies in rat show that it is distributed throughout various tissues, urine, and intestinal contents by 1 h (1 mg/kg p.o. [$^{14}$C]MGS0028, as measured by radioactivity). However, in the brain, rat whole body radioluminography only detected radioactivity in the area postrema. Subsequent studies utilizing liquid chromatography with radioactive detection measured brain (cerebrum and cerebellum), CFS, choroid plexus, blood, and plasma levels over time after administration at 1 mg/kg i.v. [$^{14}$C]MGS0028. Radioactivity was detectable in all tissues except the choroid plexus, suggesting that the blood-cerebrospinal fluid barrier is

![Fig. 7. MGS0028 and metabolite (MGS0034) plasma concentrations in Sprague-Dawley rats. Results are presented as the mean ± S.D., n = 3.](image)

![Fig. 8. MGS0028 and metabolite (MGS0034) plasma concentrations in rhesus monkeys. Results are presented as the mean ± S.D., n = 3.](image)

![Fig. 9. MGS0028 plasma concentrations in beagle dogs. Results are expressed as the mean ± S.D., n = 3. The metabolite MGS0034 was not detected in dog plasma.](image)

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<thead>
<tr>
<th>TABLE 4</th>
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**TABLE 5**

*Predicted human pharmacokinetic parameters*  

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<tr>
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<th>$V_d$</th>
<th>CL</th>
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</tr>
<tr>
<td>$V_{2a}$</td>
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<td>C3b*</td>
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<td>$V_{3a}$</td>
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<td>C3d</td>
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<tr>
<td>Average $V_d$</td>
<td>0.09</td>
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*Methods reported by Obach et al. (1997).*
The circles represent data points for log(body weight) versus log(CLp). The straight line is the plot of the predicted line from linear regression. The triangle is the calculated penetration is low when compared with plasma to MGS0028-derived compounds is very low at efficacious doses slower than that from plasma. Thus, even though total brain exposure suggesting that at high plasma concentrations, there is enough com-
ported into the brain by an amino acid transporter, specifically the glutamate transporter. Endogenous glutamate levels in brain persist at
about a 50-fold lower concentration than in plasma (1–10 μM in brain and 40–60 μM in plasma), and brain levels are maintained by both active influx and efflux transporters (Oldendorf and Szabo, 1976; Gottlieb et al., 2003). Being a glutamate analog, MGS0028 may be actively trans-
ported into the brain by an amino acid transporter, specifically the glutamate transporter. Endogenous glutamate levels in brain persist at
about a 50-fold lower concentration than in plasma (1–10 μM in brain and 40–60 μM in plasma), and brain levels are maintained by both active influx and efflux transporters (Oldendorf and Szabo, 1976; Gottlieb et al., 2003). Interestingly, MGS0028 enters the brain rapidly, when plasma levels are highest (0.1 μM in brain and 1 μM in plasma), suggesting that at high plasma concentrations, there is enough com-
compound present to compete with glutamate at these transporters.

Another important finding was that elimination from the brain is slower than that from plasma. Thus, even though total brain exposure to MGS0028-derived compounds is very low at efficacious doses (∼0.3–3 mg/kg) (Nakazato et al., 2000; Takamori et al., 2003) and penetration is low when compared with plasma Cmax (< 0.1), slow brain elimination might prolong the duration of action. Although this effect may be a benefit, it also has the unfortunate effect of undermining the ability to track pharmacodynamic readouts with plasma levels, an important consideration for future development. The slower elimination of MGS0028 from the brain than from plasma would suggest either that MGS0028 is not a substrate for glutamate efflux transporters, or that competition with endogenous glutamate results in slow efflux transport kinetics. If MGS0028 is not a substrate for efflux transporters, then its poor membrane permeability would prevent easy passage back into the blood stream, resulting in prolonged elimination from the brain.

In vivo studies revealed that MGS0028 metabolism was species-
dependent. In rats and monkeys, MGS0028 is stereospecifically me-
tabolized to a single reductive metabolite, MGS0034, which has been characterized as the 4R-isomer (Figs. 1 and 3) by comparison to authentic synthetic standards of both the 4R and 4S derivatives. Whereas in dogs, MGS0028 metabolism is not detected. To assist in the selection of preclinical safety species, in vitro metabolic studies were done using liver subcellular fractions from four species (rat, dog, monkey, and human) to correlate with the in vivo analysis. In vitro studies revealed that the reductive metabolism occurred in cytosol, but not in microsomes; thus, MGS0028 is most likely reduced to MGS0034 by a carbonyl reductase, a NADPH-dependent cytosolic enzyme (Forrest and Gonzalez, 2000; Oppermann and Meser, 2000). Carbonyl reductase is known to stereospecifically reduce xenobiotic aliphatic ketones, such as warfarin, via an acid-base mechanism (Hermans and Thijssen, 1992; Oppermann and Meser, 2000). How-
ever, further studies are required to understand the reason why MGS0028 is not metabolized in dogs (Forrest and Gonzalez, 2000).

In vivo and in vitro metabolism correlated for all three preclinical species, including the relative abundance of metabolite formed. The in vitro rank order of metabolism was found to be monkey >> rat >> human >> dog, whereas the in vivo rank order was monkey >> rat >> dog. Thus, it is predicted that the reductive metabolite will be present in humans, since the metabolite was formed in human liver cytosol incubations. Additionally, excretion studies in rat showed that the majority of radioactivity was excreted in urine as the metabolite (90% in 0- to 8-h urine post 1 mg/kg p.o. [14C]MGS0028), indicating that the primary route of elimination in rats was reductive metabolism.

As with metabolism, there were significant differences in the PK response across the three species tested. In rats and monkeys, CLp is moderate (rat, 16.6 ml/min/kg at 3 mg/kg; monkey, 8.0 ml/min/kg at 1 mg/kg), and the Vd is on the order of total body water, correlating to wide distribution (0.5 l/kg and 0.6 l/kg, respectively). However, in dogs, both CLp and Vd are low (2.8 ml/min/kg and 0.1 l/kg at 0.3 mg/kg). Oral bioavailability is good in rats and dogs (>60% in both species), but lower in rhesus monkeys (20%). Based on quantitative results, the effective t1/2 was determined to be about 1 h in all species examined; however, qualitative data indicate a longer terminal phase, representing a minor portion of the total AUC (<1%, data not shown). MGS0028 is primarily partitioned into plasma (blood to plasma ratio 0.74 and 0.64, in rat and human, respectively) and is not highly bound to plasma proteins (approximately 70% unbound in rat, dog, rhesus, and human). Thus, protein binding should not affect the pharmacological activity or highly impact prediction of human PK. Assuming t1/2 = 1 h in human, allometric scaling to human PK parameters predicts that MGS0028 will have a low Vd (~0.1 l/kg), and low CLp (~1–4 ml/min/kg). It is difficult to interpret the impact of the short effective t1/2 on the pharmacologic action of MGS0028, due to the extended t1/2 expected in brain. Further studies are needed to under-
stand brain elimination, including taking data at later time points (>6 h) and after chronic dosing.

In summary, the reductive metabolism of MGS0028 was found to be species-dependent. A single major reductive metabolite (MGS0034) was observed in rats and monkeys, whereas no metabolite was detected in dogs. In vitro metabolic studies in rat, dog, monkey,
and human liver, cytosols were consistent with in vivo results and indicate that reductive metabolism may occur in humans. Thus, it is recommended that further preclinical development of MGS0028 be done in rats and monkeys. Based on the PK parameters of MGS0028 in the three preclinical species studied (rats, dogs, and monkeys), it was predicted with allometric scaling methods that MGS0028 would have acceptable human CL\textsubscript{p} and overall PK parameters.

Acknowledgments. We thank Kazunari Sakagami (Taisho Pharmaceutical Company) for helpful synthesis and Manabu Ito (Taisho Pharmaceutical Company) for support analysis, Masayo Yamazaki (Merck Research Laboratories, West Point) for helpful discussions, and John Hutchinson (Merck Research Laboratories, San Diego) for advice and review.

References


Toxicology 144:71–81.

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