Pharmacokinetics of a Novel Antiarrhythmic Drug, Actisomide

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The pharmacokinetics of a novel antiarrhythmic drug, actisomide, were examined in the rat, dog, monkey, and human. The terminal half-life of actisomide was similar (1.15–1.89 hr) across species, regardless of dose. The total plasma clearance was higher in the monkey (13.5–16.4 mL/min/kg) than in the dog (9.01–9.32 mL/min/kg), rat (8.6–9.8 mL/min/kg), or human (6.79 ± 1.07 mL/min/kg). Excretion of the parent drug was higher in urine than in feces in the dog and rat, whereas in the monkey and human, urinary and fecal excretions of actisomide were similar. In humans, atypical plasma concentration–time curves with double peak concentrations were observed following oral doses. Systemic availability of actisomide was higher in the dog than in the rat, monkey, and human. Further, the systemic availability appeared to increase with dose in the rat and monkey. The species-dependent systemic availability appeared to be due primarily to species-dependent absorption of actisomide, and not to species-dependent first-pass metabolism, biliary excretion, and/or renal elimination. The absorption of actisomide in the rat and its in vitro uptake in CaCo-2 cells were pH dependent. The higher systemic availability of actisomide observed in the dog may be due partly to the higher pH in the gastrointestinal (GI) tract of the dog. However, the pH differences in the GI tract of the different species alone did not appear to be enough to explain the difference in systemic availability of actisomide.

KEY WORDS: actisomide; species-dependent absorption; pH effect.

INTRODUCTION

Actisomide (SC-36602) is a novel antiarrhythmic agent (Fig. 1) that blocks sodium channels in a manner similar to that of lidocaine and mexiletine (1,2). In preclinical studies, actisomide was well tolerated and had negligible negative inotropic activity in comparison to other class I antiarrhythmic agents. The present study was undertaken to evaluate the pharmacokinetics of actisomide in laboratory animals and humans after administration of the drug intravenously and orally.

EXPERIMENTAL PROCEDURE

Materials

[14C]Actisomide, unlabeled actisomide, and N-dealkylated metabolite were supplied by G. D. Searle & Co. (Skokie, IL). All chemical reagents were commercially available.

In Vivo In Situ Study Protocol

Rats. Male rats (Charles River) weighing 250 to 300 g were fasted overnight prior to drug administration. [14C]Actisomide was administered as aqueous solutions intravenously (i.v.) at doses of 8 and 32 mg/kg and orally at doses of 16 and 160 mg/kg to each group of eight rats for the radioactivity recovery and plasma concentration–time course study. Blood, urine, and fecal samples were collected from the appropriate groups at specified time intervals.

For the biliary excretion study, rats were anesthetized with ethyl ether and anesthesia was maintained by sodium pentobarbital (intrapitoneal dose of 55 mg/kg). The common bile duct was catheterized with polyethylene tubing (PE 10, Clay Adams, Parsippany, NJ). After the animals had recovered from anesthesia, [14C]actisomide was given i.v. at an 8 mg/kg dose and orally at doses of 8, 64, and 160 mg/kg. Bile, urine, and feces were collected periodically for 72 hr.

For the absorption study over a range of pH, an 8 mg/kg dose of [14C]actisomide was administered in 0.1 M phosphate buffer solutions of pH 5, 7.4, and 8.5 to three groups of three rats each. The rats which received a pH 8.5 dose solution were pretreated with Maalox (5 mL/kg) 1 hr prior to dosing.

An in situ absorption study was conducted using a single-path perfusion method reported by Sinko and Amidon (3) with minor modifications. The dose solution (0.5 mg/mL), prepared using Ringer’s solution (pH 7.5), was perfused at a flow rate of 0.15 mL/min through approximately 10 cm of the upper jejunum, ileum, and colon of three rats each. Jejunum perfusion was conducted with and without bile duct ligation. Changes in water volume in the in situ gastrointestinal (GI) tract were corrected for by using [3H]Julin.

Dog. Eight male beagle dogs weighing 7.0–12 kg were fasted overnight prior to dosing. Four dogs received i.v. doses of [14C]actisomide at 8 and 16 mg/kg in a randomized crossover manner. The remaining four dogs received unlabeled actisomide as an oral solution at a dose of 20 mg/kg. Blood, urine, and fecal samples were collected at specified time intervals.

Monkey. Four female rhesus monkeys weighing 4.7–6.7 kg were fasted overnight prior to drug administration. Each animal received [14C]actisomide i.v. doses of 8 and 16 mg/kg body weight and oral doses of 16 and 64 mg/kg in a solution in a randomized crossover manner with a washout period of at least 1 week. During the first 10 hr of the study, animals were placed in a primate chair and saline (20 mL/hr) was infused through a catheter. The animals were then transferred to stainless-steel metabolism cages. Blood, urine, and feces were collected at appropriate time intervals.

Man. Twelve healthy male subjects between 21 and 28 years of age participated in the study. A complete medical history and results of physical and laboratory examinations were obtained for each subject. These included complete blood count, urinalysis, creatinine, serum bilirubin (total), total protein, albumin, calcium, inorganic phosphorus, cho-
lesterol, uric acid, LDH, alkaline phosphatase, BUN, glucose, and SGOT. These values in all six subjects were within the normal range. No other medications besides acitsonide were taken by the subjects during the study. The six subjects received an i.v. dose of 50 mg $\left[^{14}\text{C}\right]$actisomide/person (approx. 0.8 mg/kg). For the oral study, three subjects received a dose of 400 mg unlabeled actisomide/person (approx. 5.3 mg/kg) and three subjects received 500 mg unlabeled drug (approx. 7.3 mg/kg). The i.v. dose solution was prepared by dissolving 50 mg of $\left[^{14}\text{C}\right]$actisomide (approx. 100 $\mu$Ci) in 10 mL of isotonic citrate buffer. The dose solution (10 mL) of the drug was administered as an i.v. injection over a 5-min period. The oral dosage form was a gelatin capsule containing 100 mg neat actisomide. [The bioavailability of the drug was the same as a solution or neat chemical in gelatin capsules in laboratory animals (data not shown).] The subjects were fasted approximately 10 hr prior to and 4 hr after dosing. On the day of the oral study, each subject drank 250 mL of water 1 hr before receiving the drug. An additional 50 mL of water was taken with the drug. After i.v. administration, blood samples were taken at 0 (immediately after cessation of infusion), 1, 2, 3, 4, 5, 6, 7, 10, 15, 30, and 45 min and 1, 2, 4, 6, 8, 10, 12, 16, 24, 48, and 72 hr. Urine was collected at 0, 1, 2, 3, 4, 8, 12, 24, 48, 72, 96, 120, 144, and 168 hr. Feces was collected every 24 hr up to 168 hr. After oral administration, blood samples were collected at 0, 0.25, 0.5, 0.75, 1, 1.25, 1.75, 2, 3, 4, 5, 6, 7, 8, 10, 12, 14, 24, 28, 32, 48, 52, 56, and 72 hr.

**In Vitro CaCo-2 Cell Uptake**

An in vitro uptake study of $\left[^{14}\text{C}\right]$actisomide was conducted with CaCo-2 cells (American Type Culture Collection, Rockville, MD) at pH's of 6.5, 7, and 8 and at drug concentrations of 0.1, 0.5, 1, and 5 $\mu$g/mL using a procedure reported by Ruben et al. (4).

**Sample Analysis**

**Plasma.** Total radioactivity in animal and human plasma was determined by liquid scintillation counting (LSC) after the addition of a 50–500 $\mu$L aliquot to a mixture of 2 mL of water and 5 mL of PCS scintillant (Amersham Co., Arlington Heights, IL). To quantitate the plasma concentrations of the parent drug in all species, plasma samples were extracted as follows: an aliquot of plasma (0.5–1 mL) containing an internal standard (SC-13957) was made basic by the addition of 0.5 $N$ NaOH and applied to a CN Bond Elut column (size, 3 cm$^2$; AnalytiChem International Inc., Harbor City, CA) which was preactivated with methanol followed by a water rinse. Actisomide was eluted with a solvent mixture of methanol:water:1 M dibutylamine phosphate (91:8:1, vol). The eluent was evaporated to dryness. The residue was reconstituted with a mixture of CH$_3$CN and 0.01 M dibutyl amine phosphate (90:10, vol) and analyzed using a high-performance liquid chromatographic (HPLC) procedure.

**Urine.** Total radioactivity in animal and human urine was determined by counting triplicate 0.5- to 1.0-mL aliquots directly in a mixture of 1 mL of distilled water and 5 mL of PCS scintillant. To measure actisomide concentrations, aliquots of the pooled or individual urine samples (1 mL) were made basic by the addition of 0.5 $N$ NaOH and placed in the activated Bond Elut column. Radioactive compounds were eluted from the Bond Elut column and the eluants were analyzed using a high-performance liquid radiochromatographic (HPLRC) procedure.

**Feces.** Each animal and human fecal sample was mixed with an equal weight of distilled water and the mixture was homogenized in a Stomacher (Lab-Blender 400, A. J. Seward, London). Triplicate aliquots of each homogenized sample were dried at room temperature for at least 15 hr and oxidized with a Packard Tri-Carb sample oxidizer (Model 306, Packard Instruments Co., Downers Grove, IL). The combustion products were mixed with 9 mL of Carbosorb and 12 mL of Permafluor V (Packard Instruments Co., Downers Grove, IL) and the total radioactivity was determined by LSC. To determine the distribution of radioactivity in feces, an aliquot (0.6–1.0%) was pooled from each of the selected fecal samples proportionally to its weight. The pooled fecal samples were extracted by refluxing with methanol for approximately 16 hr in Soxhlet extraction tubes. The methanol extract was applied to a Bond Elut column and eluted according to the same procedure as that described for the urine samples.

**Liquid Scintillation Counting (LSC).** All radioactivity determinations were carried out using a liquid scintillation spectrometer (Mark II or III, Tracer Analytic Inc., Elk Grove, IL). Chemical quenching was corrected by the automatic external standard channel ratio method.

**HPLC and HPLRC.** The HPLC system consisted of pumps (Waters M-6000), a system controller (Waters Associates), a WISP automatic injector (Waters Model 710B, Waters Associates), a UV detector (Kratos Spectrolow 783 absorbance detector, Kratos Analytical Instruments, Ramsey, NJ), and a reverse-phase column (Radial-Pak CN column, Waters Associates). The mobile phase, which consisted of water, acetonitrile, and 1 M dibutylamine phosphate (88:11: 1, vol), was used at a flow rate of 1.5 mL/min. Actisomide was detected at a fixed wavelength of 254 nm. HPLRC was performed using a C-18 radial compression cartridge (8-mm ID, 10-$\mu$m particle size). The mobile phase consisted of a linear gradient system from 5% acetonitrile in 0.01 M dibutylamine phosphate to 90% acetonitrile in 0.01 M dibutylamine phosphate over a 60-min period. The flow rate was 1 mL/min. Eluant fractions from the column were collected every minute in liquid scintillation vials using a Foxy fraction collector (Foxy ISCO, Lincoln, NE). Five milliliters of PCS was added to each vial and total radioactivity was determined by LSC.