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Metabolism and physiologically based pharmacokinetic modeling of flumioxazin in pregnant animals

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ABSTRACT

A physiologically based pharmacokinetic (PBPK) model was developed to predict the concentration of flumioxazin, in the blood and fetus of pregnant humans during a theoretical accidental intake (1000 mg/kg). The data on flumioxazin concentration in pregnant rats (30 mg/kg po) was used to develop the PBPK model in pregnant rats using physiological parameters and chemical specific parameters. The rat PBPK model developed was extrapolated to a human model. Liver microsomes of female rats and a mixed gender of humans were used for the in vitro metabolism study. To determine the % of flumioxazin absorbed after administration at a dose of 1000 mg/kg assuming maximum accidental intake, the biliary excretion study of [phenyl-U-^{14}C] flumioxazin was conducted in bile duct-cannulated female rats (Crl:CD (SD)) to collect and analyze the bile, urine, feces, gastrointestinal tract, and residual carcass. The % of flumioxazin absorbed at a dose of 1000 mg/kg in rats was low (12.3%) by summing up ^{14}C of the urine, bile, and residual carcass. The pregnant human model that was developed demonstrated that the maximum flumioxazin concentration in the blood and fetus of a pregnant human at a dose of 1000 mg/kg po was 0.86 μg/mL and 0.68 μg/mL, respectively, which is much lower than K_m (202.4 μg/mL). Because the metabolism was not saturated and the absorption rate was low at a dose of 1000 mg/kg, the calculated flumioxazin concentration in pregnant humans was thought to be relatively low, considering the flumioxazin concentration in pregnant rats at a dose of 30 mg/kg. For the safety assessment of flumioxazin, these results would be useful for further in vitro toxicology experiments.

Introduction

Flumioxazin [7-fluoro-6-(3,4,5,6-tetrahydrophthalimido)-4-(2-propynyl)-*2H*-1, 4-benzoxazin-3(*4H*)-one] is an N-phenylimide herbicide widely used for controlling annual broadleaf weeds in soybeans (Yoshida et al., 1991). Its herbicidal activity depends on the inhibition of the biosynthesis of porphyrin. Administration of flumioxazin at a dose of 30 mg/kg po without maternal toxicity caused embryonic lethality, teratogenicity, and growth retardation in rats but administration at the maternal toxicity level of 3000 mg/kg po did not yield the same toxicities in rabbits (Kawamura et al., 1995). The observed toxicity of anemia is thought to be associated with the inhibition of porphyrin biosynthesis, particularly the inhibition of protoporphyrinogen oxidase (PPO). Among tested species of rats, humans, and rabbits, the PPO activity in rats was most sensitively inhibited by flumioxazin (Australia APVMA, 2003).

The metabolism and disposition of ^{14}C-flumioxazin at doses of 1 and 100 mg/kg in rats have been previously reported (Tomigahara et al., 1999a,b). Administered ^{14}C-flumioxazin was completely excreted into feces (56–85%) and urine (13–43%). Seven metabolites were identified by spectroanalyses (NMR and MS), and proposed metabolic pathways are as follows: (1) hydroxylation of the cyclohexene ring; (2) cleavage of the imide linkage; (3) cleavage of the amide linkage; (4) acetylation of the amino group; and (5) sulfoconjugation of the tetrahydrophthalimide moiety.

A physiologically based pharmacokinetic (PBPK) model is a useful tool for understanding chemical disposition in humans. A lot of research has been conducted to estimate the pharmacokinetics in humans from chemical disposition data in animals (Crowell et al., 2011; Godin et al., 2010). In these studies, animal PBPK models are developed by first using animal pharmacokinetic (PK) data. Next, in vitro studies to find the relationship between animals and humans, especially in vitro metabolism studies using animal and human liver microsomes, are performed. Finally, physiological data from humans is cited from literature, and the human model is developed to predict the pharmacokinetics in humans in several tissues, including the liver, kidney, and adipose.

Abbreviations: PBPK, physiologically based pharmacokinetics; K_m, Michaelis–Menten constant; V_{max}, maximum velocity; PPO, protoporphyrinogen oxidase; NMR, nuclear magnetic resonance; MS, mass spectrometry; PK, pharmacokinetics; LSC, liquid scintillation counter; Pt, partition coefficient; Ksi, stomach-intestine transfer rate constant; Kfe, fecal excretion rate constant; Ki, intestinal uptake rate constant; Fa, fraction absorbed.

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The concentration of flumioxazin in the fetus is crucial for the elucidation of embryonic lethality, teratogenicity, and growth retardation. The study conducted in our laboratory showed that the concentration was higher in rats than in rabbits (Australia APVMA, 2003). However, it is impossible to experimentally assess the concentration in the human fetus. Although the PBPK models of herbicides in pregnant animals such as rats, mice, or rabbits have already been reported (Kim et al., 1996; Lin et al., 2013), there are no studies in pregnant humans. Thus, this report is the first case study predicting the concentration of herbicides in pregnant humans.

To estimate the concentration of flumioxazin in the human fetus, we developed a PBPK model for pregnant rats, and this model was scaled to humans. The results provide insight into the understanding of the disposition of flumioxazin in pregnant humans. Derived concentrations of flumioxazin in pregnant humans would be useful for in vitro toxicology experiments.

Methods

Chemicals. [Phenyl-U-^{14}C]flumioxazin (radiochemical purity: 98.6%) (Fig. 1) was chemically synthesized in our laboratory with a specific activity of 4.00 GBq/mmol. Unlabeled flumioxazin (chemical purity: 99.4%) was synthesized in our laboratory and used for the identification. Other chemicals were of reagent grade unless otherwise noted in the text.

In vitro metabolism of flumioxazin in female rats and human liver microsomes. [Phenyl-U-^{14}C]flumioxazin (5.6–100 μM, diluted with unlabeled flumioxazin) was incubated with 3 mM NADPH in the presence of female rat or human liver microsomes (1 mg/mL) for 20 min at 37 °C. Enzyme preparations used have been previously described (Takaku et al., 2011). All incubations were performed in 100 mM phosphate buffer (pH 7.4). Control experiments were performed without liver microsomes. After incubation, the reaction was terminated by adding an equivalent volume of ice-cold acetonitrile. Subsequently, the mixtures were stored on ice for 10 min and centrifuged at 10,000 ×*g* for 5 min. Nine parts of supernatant were mixed with one part of acetic acid and analyzed by Thin Layer Chromatography (TLC) (see the TLC analysis section).

The Michaelis–Menten constant (K_m) and maximum velocity (V_{max}) for flumioxazin disappearance were calculated using the Hanes–Woolf Plot. In the Hanes–Woolf Plot, the slope is shown as $1/V_{max}$, and the intercept is shown as K_m/V_{max} (Table 1).

Test animals and housing. All animal experiments were conducted in accordance with The Guide for the Care and Use of Laboratory Animals in the Environmental Health Science Laboratory of Sumitomo Chemical Co., Ltd. Adult female bile duct-cannulated Sprague–Dawley (Crl:CD (SD)) rats were obtained from Charles River Laboratories (Kanagawa, Japan). Since animals were received surgically instrumented, no acclimation period was used. After receipt, animals were weighed and identified by marking the tail end of each rat with a unique animal ID number using a permanent marker. During the collection periods, animals were fed pelleted diet, CRF-1 (Oriental Yeast Co., Ltd., Tokyo) ad libitum and given filtered tap water that was passed through a water filtration device ad libitum, which was supplied in a water bottle.

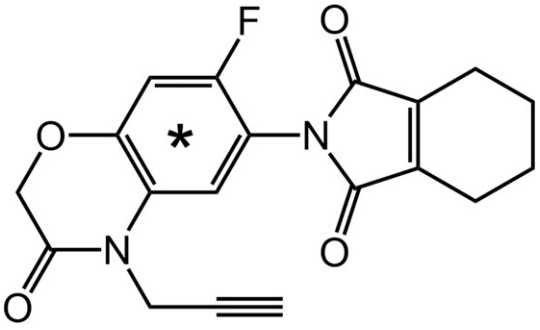

Fig. 1. Chemical structure of [phenyl-U-^{14}C]flumioxazin. *represents the position of ^{14}C.

Table 1
The kinetic parameters for [phenyl-U-^{14}C]flumioxazin degradation in female rat and human liver microsomes.

	Female rat	Human
K_m (mg/L)	34.8	202.4
V_{max} (mg/h/kg)	84.7	207.8
V_{max}/K_m (L/h/kg)	2.4	1.0

Preparation of dose solutions. Dose solutions were prepared with isotopically diluted [phenyl-U-^{14}C]flumioxazin so that each animal received a target dosage of 3.7 MBq/kg. Isotopic dilutions were performed with the radiolabeled and unlabeled test substances dissolved in acetonitrile to assure homogeneity. After isotopic dilution (3.7 kBq/mg), the solution was concentrated to near dryness under a stream of nitrogen and [phenyl-U-^{14}C]flumioxazin was redissolved in a dosing vehicle (corn oil). The dose preparation was mixed thoroughly using a mortar and pestle to ensure homogeneity. In order to measure the radiochemical purity of labeled test compounds in the dose solutions, aliquots of each dose solution were taken after the final administration and dissolved in acetone for TLC analysis (see the TLC analysis section). The results indicated that the radiochemical purities of labeled test compounds in the dose solutions were ≥98.64%.

^{14}C-bile study. The study had a single dose group consisting of 3 female non-fasted, bile duct-cannulated rats. Gavage administration was performed with a glass syringe with a stopper, equipped with a stainless-steel ball-tipped catheter. The target dose was 1000 mg/kg body weight, and the target dose volume was 5 mL/kg body weight. Bile, urine and feces samples were collected from each animal at approximately 0–24, 24–48 and 48–72 h post-dose. After collecting urine, the inside of the cage was washed twice with approximately 100 mL of water, and the cage wash was collected. Each rat was euthanized and the gastrointestinal tract was collected at 72 h post-dose. The contents of the gastrointestinal tract were removed and sampled, and the remaining tract was combined with the carcass to make the residual carcass. Radioactivity in each sample obtained was measured according to the method described in the Radioanalysis section. The radioactivity in the cage wash was considered to be excreted via the urine. Absorption was calculated by summing up the urinary and biliary ^{14}C-excretion and ^{14}C in the carcass.

Radioanalysis. The radioactivity in urine, bile and organosoluble fractions was measured by a liquid scintillation counter (LSC) (TRI-CARB 2500TR, PerkinElmer Japan). About 0.2 g of fecal homogenates, unextractable fecal residues, and tissues were combusted using an oxidizer, and radioactivity was measured by LSC.

TLC analysis. Pre-coated silica gel 60 F 254 TLC plates (20 × 20 cm, 0.25 thickness, Merck, Germany) were utilized along with chloroform/toluene/ethanol/acetic acid, 18:2:1:1 (v/v/v/v) and toluene/ethyl acetate/acetic acid, 3:6:1 (v/v/v), as a solvent system to determine the radiochemical purity of ^{14}C-flumioxazin. In addition, toluene/ethyl acetate/acetic acid, 50:50:1 (v/v/v) was used to analyze the samples for in vitro metabolism. Radioactive compounds on TLC plates were detected by autoradiography using imaging plates (Fuji Photo Film, Tokyo, Japan). These plates were contacted with TLC plates at room temperature and then processed with a fluorescent image analyzer (FLA-5000, Fuji Photo Film).