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Metabolic disposition and excretion of quinocetone in rats, pigs, broilers, and carp

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ABSTRACT

Excretion, disposition, and metabolism of [^{3}H]-quinocetone in rats, pigs, broilers, and carp following oral administration were investigated. After a single p.o. dose, total radioactivity was rapidly excreted, with ⩾94% in all species within 14 days. Fecal excretion of radioactivity was 68% and 65% of the administered dose in rats and pigs, respectively, with the remainder excreted in the urine. Six hours after the last of seven daily oral administrations of ^{3}H-labeled QCT, radioactivity was found to be distributed throughout all tissues, with the majority of radioactivity cleared within 7 days, and elimination was the slowest from the liver and kidney. QCT was extensively metabolized in all of the species, and the primary changes included N–O group reduction, carbonyl group reduction, double bond reduction, and hydroxylation. The major tissue metabolites of QCT were Q2, Q4, Q5, Q8, and Q9 in rats; Q1, Q2, Q3, Q4, and Q5 in pigs; Q1, Q2, Q3, Q4, and Q7 in broilers; and Q1, Q2 in carp. This confirmed the potential link between QCT metabolism through N–O group reduction and its organ toxicity. The results of the present study provide important data that could help understand the relationship between the toxicities and metabolic disposition of QCT.

1. Introduction

Quinocetone (QCT; Fig. 1), 3-methyl-2-quinoxalinbenzenevinylketo-1,4-dioxide, an antimicrobial agent, was approved by Ministry of Agriculture, PR China in 2003. It is extensively used as a feed additive for preventing bacterial infections caused by *Salmonella, Escherichia coli, Brachyspira hyodysenteriae* and other gram-negative bacteria, and for promoting growth and feed conversion. Nevertheless, recently conducted studies have identified the adverse effects of QCT both *in vitro* and *in vivo*, such as genotoxicity, hepatotoxicity and nephrotoxicity (Ihsan et al., 2013; W. Yang et al., 2013; X. Wang et al., 2012, 2010; D. Wang et al., 2011, 2012; Yu et al., 2013). Drug metabolism and disposition generally regulates the pharmacodynamic, toxicological, and pharmacological effects of many drugs significantly, in terms of food-producing animals, metabolism also defines the residue profile of both parent drugs and metabolites in edible tissues (Antonovic and Martinez, 2011), thus determining the quality of such food animals, a major concern for the human health safety. Moreover, previous studies showed that the quinoxalines, such as carbadox (CBX) and olaquindox (OLA) were converted to several metabolites that were closely associated with their toxicities (Chen et al., 2009; FAO/WHO, 1995, 1990), particularly with the production of reduced metabolites (Beutin et al., 1981). Because QCT shares considerable structural similarity with CBX and OLA, QCT was proposed to be metabolized

Abbreviations: QCT (Q0), quinocetone; Q1, 1-desoxyquinocetone; Q2, dideoxyquinocetone; Q3, carbonyl-reduced metabolites of 1-deoxyquinocetone; Q4, carbonyl- and double-bond-reduced metabolites of 1-deoxyquinocetone; Q5, carbonyl-reduced metabolites of dideoxyquinocetone; Q6, phenyl ring hydroxylation metabolites of carbonyl-reduced quinocetone; Q7, carbonyl-reduced quinocetone; Q8, double-bond-reduced metabolite of 4-deoxyquinocetone; Q9, phenyl ring hydroxylation metabolites of quinocetone; OLA, olaquindox; CBX, carbadox; QdNOs, quinoxaline 1,4-dioxides; MQCA, 3-methylquinoxaline-2-carboxylic acid; MEQ, mequindox; QCA, quinoxalie-2-carboxylic acid; CYA, cyadox; JECFA, Joint FAO/WHO Expert Committee on Food Additives; VICH, International Cooperation on Harmonization of Technical Requirements for the Registration of Veterinary Medicinal Products; LSC, liquid scintillation counting; EIC, extracted ion chromatograms; CYP, cytochrome P450 enzyme; CBR, carbonyl reductase; H_2O_2, hydrogen peroxide; ROS, reactive oxygen species.

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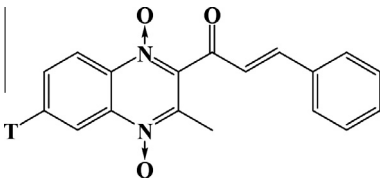

Fig. 1. Chemical structure of [^{3}H]-QCT.

by the same biochemical pathways as those for these drugs. 1,4-Bisdesoxyquinocetone was less toxic to liver cells than QCT (Zhang et al., 2012). QCT toxicities are apparently dependent on its metabolic pathway; however few studies were conducted on QCT disposition in laboratory animal (e.g., rats) and in food animals (e.g., pigs, broilers, and carp).

Studies have previously been conducted to examine the residual depletion of QCT in pigs, chickens, and carp, in which liver was the target tissue, 3-methylquinoxaline-2-carboxylic acid (MQCA) (Hu et al., 2008) or 3-methyl-styrylketone-quinoxaline (Zhong et al., 2012) was the marker residue, the withdrawal time of QCT was recommended as 0 days (Li et al., 2008). However, these previous studies primarily focused on QCT and a few of its known metabolites. *In vitro* and *in vivo* QCT metabolism was reported recently. A previous study showed QCT metabolism in the liver microsomes of rats by LC/MS-IT-TOF, and identified 27 metabolites (Liu et al., 2010a). *In vivo* QCT metabolism in pigs was investigated by UPLC/Q-TOF-MS, and 42 metabolites were found (Wu et al., 2012). However, tissue metabolites and quantitative analysis of the metabolite profiles were not reported.

Therefore, there is an immediate need to gain a complete understanding of the metabolic pathways, disposition kinetics, and excretion characteristic of QCT to assess the potential toxicities and food safety of this compound. According to the recommendation by JECFA, we conducted isotopic tracing studies following the guidelines of VICH (2010). We here undertook a comparative study (both quantitative and qualitative) of the metabolite profiles of QCT in rats, pigs, broilers, and carp following oral administration. Moreover, we reported the rates and routes of excretion of radioactivity of [^{3}H]-QCT in the urine, and feces (the mass balance for excretion). In addition, we presented QCT distribution in tissues after repeated intragastric administration of [^{3}H]-QCT. The present study makes contribution to improve our understanding of the food safety and the relationship between the toxicities and metabolic disposition of QCT.

2. Materials and methods

2.1. Chemicals

4-[^{3}H]-o-nitroaniline (116 mCi/mg) was synthesized by the Shanghai Institute of Applied Physics, Chinese Academy of Sciences. QCT (99.8%) was purchased from China Institute of Veterinary Drug Control (Beijing, China). Four putative metabolites, Q1, Q2, Q3, and Q5 were synthesized at the Institute of Veterinary Pharmaceuticals (Wuhan, China), with the chemical purity of 98%. Solvable™ (tissue-solubilizing fluid), Ultima Gold and monophase A (Liquid scintillation cocktails) were purchased from PerkinElmer Life and Analytical Sciences (Groningen, The Netherlands; Waltham, MA, USA). Stop Flow™ AD scintillation liquid was obtained from the AIM Research Co. (Hockessin, DE, USA).

To a cooled stirring solution of 4-[^{3}H]-o-nitroaniline (40 mg) and solid sodium hydroxide (16 mg) in isopropanol (240 μL), sodium hypochlorite solution (600 μL, containing 5% available chlorine) was added dropwised. The mixture was stirred at 20 °C for 4 h and filtrated to give 4-[^{3}H]-benzofurazan-1-oxide (Xmg). After it was dissolved in methanol (100 μL), acetylacetone (150 μL) and triethylamine (400 μL) were added. The solution was then stirred for 15 h at r.t. to obtain 6-[^{3}H]-3-methyl-2-acetyl-N-1,4-dioxyquioxaline, which was applied to a aldol condensation reaction with benzaldehyde to give the desired products: [^{3}H]-QCT (Fig. 1). The final labeled product was reconstituted in ethanol to provide 210 mCi [^{3}H]-QCT (yield, 21%), with a radiochemical purity of 99%. Specific activity was 42 mCi/mg, and the specific activities were diluted to 11.25 mCi/g for the animal study. Exchange of the ^{3}H label with water was confirmed.

2.2. Animals

Male and female specific pathogen-free Wistar rats (*Rattus norvegicus*, 7 weeks, 200 ± 10 g), were procured from the Center of Laboratory Animals of Hubei Province (Wuhan, PR China). Healthy castrated crossbred (Duroc × Large white × Landrace) pigs (45 days, 15 ± 1 kg) were purchased from the Livestock and Poultry Breeding Center of Hubei Province (Wuhan, PR China). Healthy Cobb 500 broilers (28 days, 1.0 ± 0.1 kg) were purchased from the Charoen Pokphand Group (Wuhan, PR China), and healthy carp (500 ± 100 g) were purchased from the Wuhan Fish Breeding Farm (Wuhan, PR China). The animals and fish were maintained under standard environmental conditions using the routine methods of animal husbandry and aquaculture. Throughout the study period, feed was withheld from approximately 12 h before until 4 h after drug administration, while water was available *ad libitum*. The animals were housed singly in all-steel metabolism cages specifically designed for the separate, quantitative collection of urine and feces in a temperature-controlled room (20 ± 2 °C) with a 12-h light/dark cycle. The Ethical Committee of the Faculty of Veterinary Medicine (Huazhong Agriculture University) approved the present study. All in-life experiments complied with the policy on the care and use of laboratory animals.

2.3. Clinical study design

2.3.1. Excretion and metabolism of [^{3}H]-QCT

Three male and three female rats (four pigs, six broilers, and six carp) were administered a single oral dose of [^{3}H]-QCT (11.25 mCi/g) by gavage at 4 mg ± 0.4 mg (120 mg ± 8 mg, 20 mg ± 2 mg, and 2.5 mg ± 0.5 mg, respectively, for the other animals) equivalent of 200 mg/kg diet. Urine and feces samples were collected into preweighed dry ice-cooled containers protected from light prior to dose administration; at intervals of 0–6 h, 6–12 h, and 12–24 h after dose administration; and then daily until two consecutive samples from urine and feces had <1% of the total administered radioactivity. The cage washings were retained with the excreta. Fourteen days after dose administration, all animals were anesthetized and killed, and pigs were slaughtered using a captive bolt stunning equipment and exsanguinated on the basis of guidelines provided by the American Veterinary Medical Association for euthanasia (AVMA, 2001). Samples were stored at −80 °C prior to analysis.

2.3.2. Distribution of [^{3}H]-QCT

Each of the 36 rats (20 pigs, 36 broilers, and 36 carp) were given 2 mg ± 0.2 mg (60 mg ± 4 mg, 10 mg ± 1 mg, and 1.25 mg ± 0.25 mg, respectively, for the other models) [^{3}H]-QCT (11.25 mCi/g) equivalent of 100 mg/kg diet by oral gavage for 7 consecutive days. At different time points [6 h, 2, 5, 9, 14, and 21 days (6 h, 1, 3, 7 and 14 days for the other animals)], six rats (four pigs, six broilers, and six carp) were anesthetized and killed, and pigs were slaughtered as described in Section 2.3.1. Blood, bile, organs and tissues (gastrointestinal tract, kidneys, liver, heart, spleen, lung, muscle, fat, skin, brain, gonad, adrenal gland, and bladder) were sampled. Samples were immediately frozen at −80 °C.

2.4. Analysis of total radioactivity

Duplicate samples were analyzed by liquid scintillation counting (LSC) until a statistical error (2σ) of 0.5% was obtained, with a counting time of 5 min, together with representative blank samples. LSC was performed using a Packard Tri-Carb 2900 liquid scintillation analyzer with automatic quench correction by an external method (PerkinElmer Life and Analytical Sciences, Waltham, MA, USA) to detect retained ^{3}H radioactivity. To calculate the content of volatile radioactivity such as ^{3}H water, another duplicate set of samples was freeze-dried and reconstituted in water (“dry samples”) prior to analysis. 200 μL of the urine samples were diluted in 10 mL monophase A and subjected to LSC analysis. Feces samples were homogenized with a mixer (Omni International, USA) in equal amount of methanol/water (50/50, v/v). Subsequently, 200 mg of the homogenized samples were incubated overnight at 55 °C with 2 mL of sodium hypochlorite and subjected to LSC analysis. Before LSC analysis, whole blood (200 μL) and organ (200 mg) samples were incubated overnight at 55 °C with 2 mL of Solvable™. After incubation, the samples were decolored with 200 μL of 0.1 mol/L EDTA-Na and 200 μL of hydrogen peroxide (30%) (500 μL in case of spleen) and counted. The efficiency of digestion was confirmed by digestion of radiochemical standards (^{3}H-Spec-Chec; PerkinElmer Life and Analytical Sciences, Waltham, MA, USA) for every 10–15 samples and was between 96% and 98%. A correction factor was used accordingly. All samples were prepared in 10 mL scintillation fluid (Ultima Gold) and incubated at room temperature overnight in a dark environment prior to LCS analysis.