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Tissue Deposition and Residue Depletion of Cyadox and Its Three Major Metabolites in Pigs after Oral Administration

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ABSTRACT: Tissue deposition and residue depletion profiles of cyadox (Cyx) and its three major metabolites, including 1,4-bisdesoxycyadox (Cy1), 4-desoxycyadox (Cy2), and quinoxaline-2-carboxylic acid (QCA), in pigs after multiple oral administrations were determined. Thirty-five healthy adult pigs were randomly divided into seven groups and orally treated with Cyx at a dosage of 20 mg/kg of body weight for five consecutive days. Each group of five pigs was randomly slaughtered 12, 24, 72, 120, 168, 216, and 264 h after the last dosing, and tissue samples, including muscle, liver, kidney, and fat, were collected and analyzed via the liquid chromatography−tandem mass spectrometry method. The concentration−time data of Cyx and its three metabolites (Cy1, Cy2, and QCA) were analyzed with WinNonlin. Results showed that metabolites of Cyx were quickly generated in swine tissues and the concentrations of QCA in kidney were higher than those of Cyx and other metabolites in all edible tissues. These results provide further insight into the metabolism of Cyx and confirmation of the residue marker and target tissue of Cyx in pigs.

KEYWORDS: *cyadox, residue, elimination, metabolites, pig*

INTRODUCTION

Cyadox [Cyx (Figure 1)], a new derivative of quinoxaline *N*-dioxides, was used as a feed additive in Eastern Europe in the past.[1,2] Some evidence demonstrated that the mechanism of Cyx for improving pig growth performance was correlated with several metabolic hormones and growth factors.[3] In addition, a long-term toxicity test, a subchronic oral toxicity test, and a phototoxicity test of Cyx showed that Cyx was much safer than olaquindox (OLA).[4−6] All available data indicate the potential effect of Cyx in animals is promising and Cyx is superior to other well-known quinoxalines such as olaquindox and carbadox (CBX), which have been banned or strictly limited in their use in food-producing animals because of their potential toxicities.[7] Hence, it is hopeful that Cyx would be developed as a replacer of olaquindox and carbadox with greater safety and excellent effectiveness in growth promotion and antibacterial activity. There is a possibility that Cyadox will be considered for registration in China after comprehensive pharmacological and toxicological research.

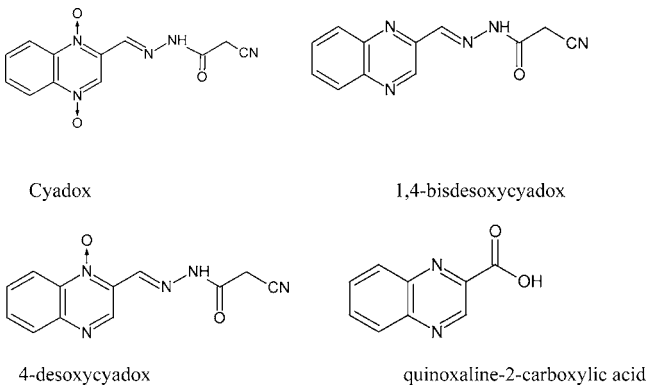

Figure 1. Chemical structure of cyadox and its three major metabolites.

Only recently have several aspects of the metabolism of Cyx been studied extensively.[8−11] The results demonstrated that Cyx could be rapidly metabolized and converted into a variety of metabolites. Three main metabolites, 1,4-bisdesoxycyadox (Cy1), 4-desoxycyadox (Cy2), and quinoxaline-2-carboxylic acid (QCA), have been reported previously. The chemical structures of these metabolites are shown in Figure 1.

Although an increasing number of methods have been developed for the detection of Cyx and its metabolites in several food-producing animal species such as goats, pigs, chickens, and fish,[12−16] to the best of our knowledge, currently, there is little information about the deposition and residue depletion of Cyx and its three major metabolites (Cy1, Cy2, and QCA) in animals. To improve our understanding of the actual metabolism of Cyx in food-producing animals, in a

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continuation of our effort to study the metabolic characteristics of Cyx in many animals, the objective of this research was to describe deposition and residue depletion of Cyx and its major metabolites (Cy1, Cy2, and QCA) in edible tissues of pigs after multiple oral administrations.

MATERIALS AND METHODS

Chemicals and Drugs. The Cyx reference standard (98.0% pure) was provided by the College of Veterinary Medicine of Huazhong Agricultural University (Wuhan, China). Metabolites Cy1, Cy2, and QCA of >97% purity were supplied by the College of Veterinary Medicine of the China Agriculture University (Beijing, China). Methanol (MeOH), acetonitrile (ACN), and formic acid (FC) of chromatographic purity were purchased from Fisher Scientific Co. Other organic solvents such as ethyl acetate, chloroform, concentrated hydrochloric acid, and dimethyl sulfoxide (DMSO) were purchased form Guangzhou Chemical Reagent Co., Ltd. Doubly distilled water for preparing solutions was made by Milli-Q synthesis (Millipore, Bedford, MA).

Solution Preparation. Standard 100 μg/mL stock solutions of Cyx, Cy1, Cy2, and QCA were individually prepared by dissolving appropriate standard reference ingredients in DMSO and kept in −20 °C in the dark for 3 months. Mixed standard 10 μg/mL working solutions containing Cyx, Cy1, Cy2, and QCA were prepared from four standard stock solutions and held at 4 °C in the dark no longer than 1 month. A 40 mg/mL Cyx suspension was prepared by suspending moderate Cyx raw material into a 0.5% sodium carboxymethyl cellulose aqueous solution before administration by gavage.

Animals and Sampling. Thirty-five castrated pigs (healthy Duroc × Landrace × large white hybrid pigs) were randomly allocated to seven treatment groups, each containing five animals. Five additional pigs were left untreated as controls in this study. Animals were purchased from Guangzhou Lizhi Agricultural Co., Ltd., and weighed 50.0 ± 5.1 kg. The pigs were housed in seven 5 m × 6 m pens and had access to tap water and diet without any antimicrobial agent. The animal house were maintained at a constant level of 25 ± 2 °C and 50−60% relative humidity. Each pig was identified by a numbered ear tag and acclimatized for at least 7 days prior to administration.

All animals in the treatment group received an oral dose of a Cyx suspension through a stomach tube at a dose of 20 mg/kg of body weight twice daily (at approximately 8:00 a.m. and 6:00 p.m.) for five consecutive days. Diet free of drugs and water were available after administration. Each treatment group of five pigs was randomly slaughtered 12, 24, 72, 120, 168, 216, and 264 h after the last dosing, and samples of liver, kidney, muscle, and fat (subcutaneous fat) were collected. Then the samples were immediately homogenized and frozen at −20 °C until they were analyzed. All procedures were conducted in accordance with Institutional Animal Care and Use Committee protocols at the South China Agricultural University.

Analysis of Cyx and Its Major Metabolites in Edible Tissues. Sample extraction and high-performance liquid chromatography−tandem mass spectrometry (HPLC−MS/MS) conditions were adopted from the method reported by our laboratory.[16,17] A 2.0 g sample was weighed into a 15 mL polypropylene centrifuge tube. A total of 5 mL of ethyl acetate (EA) was added to the tube to extract the free Cyx and its metabolites; the tube was sonicated for 10 min and then centrifuged at 8000 rpm for 10 min at 4 °C. The residues were re-extracted with 5 mL of EA, and all supernatants (EA layer) were combined. A total of 1 mL of water and 100 μL of 12 mol/L hydrochloric acid were added to the first tube (tissue residues), and the tube was vigorously vortexed for 1 min and placed in a water bath at 90 °C for 1 h (hydrolyzing the bound and conjugated metabolites). Then, the acidic solution was back-extracted by being vortex-mixed for 2 min with 5 mL of an acetonitrile/chloroform (ACN/CF) solution [1:4 (v/v)], and the tube was centrifuged at 10000 rpm for 10 min at 4 °C. All the organic extracts were combined and evaporated to dryness under a stream of nitrogen at 40 °C. The residue was dissolved in 6 mL of a MeOH/water solution [1:5 (v/v)], defatted with 3 mL of *n*-hexane twice, and finally purified with an Oasis SPE MAX cartridge. Finally, 1 mL of eluent containing a FA/ACN mixture [2:98 (v/v)] was centrifuged at 15000 rpm for 5 min, and the supernatant was passed through a 0.22 μm filter for HPLC−MS/MS analysis, with the detection performed in negative ionization mode with a dwell time of 200 ms. The multiple-reaction monitoring (MRM) settings for confirmation of Cyx, Cy1, Cy2, and QCA in swine tissues by LC−MS/MS are listed in Table 1.

Table 1. Precursor and Product Ion Transitions Used for the Quantitative and Confirmatory Analysis of Cyx and Three Major Metabolites

compound	precursor ion (*m*/*z*)	production (*m*/*z*)	declustering potential (V)	collision energy (V)	dwell time (ms)
Cyx	270.1	65.9[a]	−54	−25	200
		175	−54	−18	200
Cy1	238.0	65.9[a]	−58	−25	200
		142.9	−58	−23	200
Cy2	253.9	158.9[a]	−65	−20	200
		186.9	−65	−17	200
QCA	173.0	101.8[a]	−45	−25	200
		146.9	−45	−13	200

[a]Presents the quantitative ion.

Standard Curve and Linear Range. An aliquot of 2.0 g of blank homogenized tissues was weighted accurately into 50 mL centrifuge tubes, and a series of 100 μL mixed standard solutions were added to obtain spiked samples in which the concentrations of Cyx and its metabolites all ranged from 2 to 200 ng/g. After being vortexed for several minutes and standing for 30 min, the samples were extracted and purified with the method mentioned above for further analysis. The standard curve regression equation and correlation coefficient were estimated via linear regression using the resultant drug chromatographic peak area (X) and the corresponding drug concentration (Y). The concentrations exceeding the highest calibration concentration were detected after dilution with various matrices.

Recoveries and Coefficients of Variation. To assess the precision and accuracy of the assay, five replicates at three different spiked concentrations (5, 50, and 200 ng/g for tissues) were tested for three consecutive days to evaluate the coefficients of variation and recoveries.

Pharmacokinetics of Cyx and Its Major Metabolites. Average tissues concentrations at each sampling time were calculated, and then concentration−time data of Cyx, Cy1, Cy2, and QCA in liver, kidney, muscle, and fat tissues were analyzed with WinNonlin (version 6.1, Pharsight Corp., Mountain View, CA) using noncompartmental methods.

RESULTS

HPLC−MS/MS Method Validation. Under analytical conditions used in this experiment, the calibration curves of Cyx, Cy1, Cy2, and QCA in four tissues ranging from 5 to 200 ng/g were selected. The correlation coefficients (R^2) of standard curves were >0.99 for the four chemical compounds. On the basis of signal-to-noise ratios of >3 and >10, the limits of detection (LOD) and quantification (LOQ) were 2 and 5 ng/g in tissues, respectively. Blank samples spiked with three different concentrations of Cyx, Cy1, Cy2, and QCA were used to evaluate the accuracy and precision of the method. Recoveries of Cyx and its three main metabolites in various matrices ranged from 84.64 to 95.08%, with inter- and intraday coefficients of variation (CVs) between 2.96 and 8.75%, as reported in Table 2. Typical LC−MS chromatograms of blank

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Table 2. Mean Recoveries and Precisions of Cyx and Its Three Main Metabolites in Fortified Tissue Samples (n = 3)

sample	spike level (ng/g)	compound	recovery (%)	intraday CV[a] (%)	interday CV[a] (%)
liver	5	Cyx	93.65 ± 2.91	3.11	4.14
		Cy1	93.67 ± 3.90	4.16	5.88
		Cy2	93.09 ± 2.97	3.19	3.45
		QCA	91.24 ± 4.17	4.57	5.82
	50	Cyx	90.13 ± 4.45	4.94	5.49
		Cy1	90.59 ± 7.05	7.78	8.75
		Cy2	90.16 ± 4.24	4.70	4.85
		QCA	91.98 ± 4.07	4.43	5.40
	200	Cyx	92.50 ± 4.50	4.87	5.03
		Cy1	89.50 ± 5.90	6.59	7.53
		Cy2	91.08 ± 5.09	5.59	8.48
		QCA	92.28 ± 4.45	4.82	5.50
kidney	5	Cyx	84.64 ± 2.53	2.99	3.25
		Cy1	88.77 ± 4.09	4.60	5.38
		Cy2	88.80 ± 3.55	4.00	5.25
		QCA	94.01 ± 3.69	3.92	6.16
	50	Cyx	95.08 ± 3.00	3.14	4.83
		Cy1	91.88 ± 7.03	7.65	7.81
		Cy2	93.55 ± 7.36	7.87	8.02
		QCA	92.49 ± 4.80	5.19	5.92
	200	Cyx	92.06 ± 3.17	3.44	3.70
		Cy1	91.04 ± 3.02	3.32	3.77
		Cy2	89.69 ± 5.67	6.32	6.69
		QCA	88.86 ± 5.21	5.86	6.86
muscle	5	Cyx	88.93 ± 4.30	4.83	5.06
		Cy1	93.78 ± 5.29	5.64	6.63
		Cy2	89.40 ± 4.53	5.07	6.12
		QCA	89.30 ± 2.90	3.25	5.01
	50	Cyx	85.48 ± 3.49	4.08	4.22
		Cy1	90.82 ± 2.69	2.96	4.19
		Cy2	91.37 ± 4.63	5.07	5.12
		QCA	91.87 ± 4.77	5.19	6.05
	200	Cyx	92.96 ± 5.47	5.88	6.29
		Cy1	93.74 ± 4.27	4.56	5.77
		Cy2	92.38 ± 4.59	4.97	5.16
		QCA	93.79 ± 3.55	3.78	5.73
fat	5	Cyx	89.46 ± 3.86	4.32	7.92
		Cy1	86.55 ± 4.99	5.76	5.82
		Cy2	90.42 ± 3.68	4.07	4.94
		QCA	89.38 ± 4.92	5.50	5.58
	50	Cyx	91.81 ± 4.69	5.11	7.01
		Cy1	89.35 ± 5.66	6.33	7.14
		Cy2	92.23 ± 5.73	6.21	6.60
		QCA	92.14 ± 5.59	6.07	7.33
	200	Cyx	91.41 ± 5.19	5.68	6.09
		Cy1	93.58 ± 3.31	3.54	5.03
		Cy2	90.07 ± 4.34	4.82	5.83
		QCA	86.82 ± 3.37	3.88	4.12

[a]CV, coefficient of variation.

liver and liver fortified with Cyx, Cy1, Cy2, and QCA are shown in Figure 2. (Representative chromatograms of Cyx, Cy1, Cy2, and QCA in kidney, muscle, and fat are not shown.) The HPLC−MS/MS analytical method here demonstrated a satisfactory applicability in the simultaneous determination of the four compounds.

Residue Depletion Study. In the residue depletion study, the edible swine tissues were collected from 35 treated pigs and the concentrations of all samples for the four analytes were quantified. The measured average concentrations for Cyx, Cy1, Cy2, and QCA in the four tissues for each group are listed in Tables 3−6. The concentrations of Cyx, Cy1, and Cy2 in liver, kidney, muscle, and fat for all pigs for the 216 h time point were either below the limit of quantification or not detected, and thus, the data are not listed in Tables 3−6. On the basis of a comparison of concentrations of the four compounds in various tissues, the highest levels of residue of Cyx and Cy2 were measured in the muscle and the highest concentrations were 61.31 ± 4.41 and 112.67 ± 7.44 ng/g for Cyx and Cy2, respectively. In a comparison with other tissues, the residual times of Cyx and Cy2 were also longer, and they still could be measured in muscle 168 h after the last oral administration; however, the residue concentrations of Cyx and Cy2 in liver and kidney were lower and could not be detected 120 h after the last dosing. For Cy1 and QCA, the highest concentrations were found in kidney, 374.28 ± 82.68 and 537.52 ± 119.32 ng/g, respectively. A longer withdrawal time was also found in kidney for Cy1 and QCA. In addition, it could be observed that the highest concentrations of Cy1, Cy2, and QCA occurred 12 h after the last administration. However, concentrations of metabolites, including Cy1, Cy2, and QCA, in fat were relatively lower. At all time points, the residue with the longest residual time in biological samples was QCA, and it still could be detected 216 h after the last administration, albeit at a low concentration (7.68 ± 1.38 ng/g). Tables 3−6 illustrate the elimination of Cyx, Cy1, Cy2, and QCA in liver, kidney, muscle, and fat of pigs.

Pharmacokinetics of Cyx, Cy1, Cy2, and QCA in Tissues. The pharmacokinetic parameters are listed in Table 7. It was remarkable that areas under the concentration−time curve (AUCs) of Cyx and Cy2 were relatively larger in muscle (4482.11 and 5861.02 h ng g^{-1}, respectively); however, AUCs of Cy1 and QCA in kidney were large (15351.12 and 28657.33 h ng g^{-1}, respectively). The longest and shortest depletion half-lives ($t_{1/2\beta}$) of Cyx were observed in muscle (49.05 h) and kidney (26.08 h), respectively. A comparison of the residue elimination times of Cyx, Cy1, Cy2, and QCA in the four tissues showed that the Cyx and Cy2 in muscle were eliminated slowly; however, Cy1 and QCA in liver and kidney were eliminated slowly, the $t_{1/2\beta}$ values being 29.84 and 35.02 h, respectively.

DISCUSSION

At present, an increasing number of studies about quinoxaline in animals have been conducted by domestic and overseas researchers. Most of those reports laid particular emphasis on the toxicity, metabolism, and detection of this class compound. Information about the pharmacokinetics of this class compound is limited, with only a few cases reported in the literature.[18,19] Residue depletion profiles of some quinoxaline compounds were determined abroad such as those of carbadox and olaquindox.[20−22] Lauridsen et al.[22] found that the level of carbadox decreased rapidly to the LOD in edible tissues 6 h after the last administration when pigs were fed with carbadox at a dose of 3.5 mg/kg of body weight for three consecutive weeks and the level of QCA was mainly distributed in tissues 24 h after the last administration. When the pigs were fed carbadox at a dose of 50 mg/kg of body weight for 1 week, the concentrations of carbadox and QCA decreased to 2 ng/g 72 h after the last administration.

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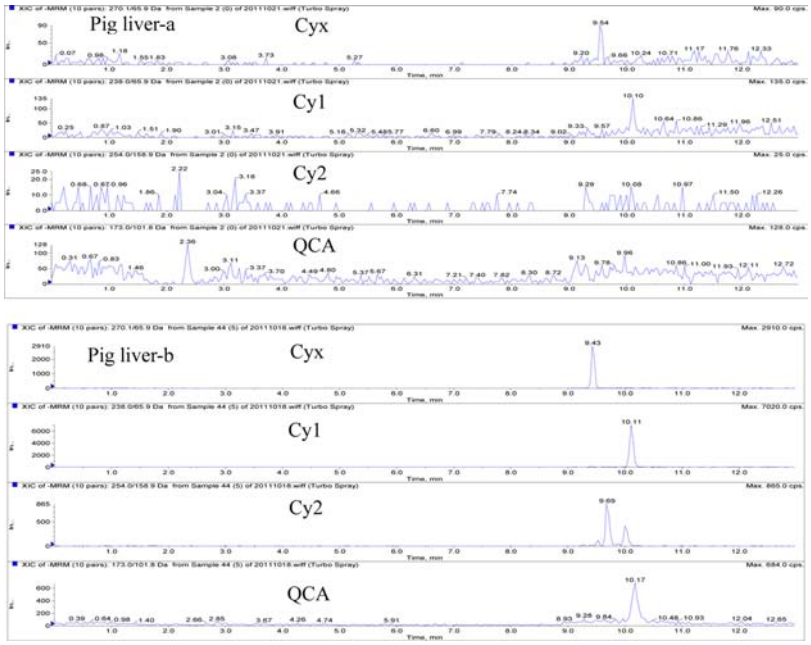

Figure 2. Representative chromatograms of swine samples fortified with Cyx, Cy1, Cy2, and QCA in (a) blank liver and (b) blank liver spiked with Cyx, Cy1, Cy2, and QCA (5 ng/g each).

Table 3. Average Concentrations of Cyx, Cy1, Cy2, and QCA in Muscle after Multiple Oral Administrations of Cyx at a Dose of 20 mg/kg of Body Weight for Five Consecutive Days

	concentration[a] (ng/g)			
time (h)	Cyx	Cy1	Cy2	QCA
12	21.58 ± 5.03	68.91 ± 14.29	112.67 ± 16.64	57.58 ± 6.39
24	61.31 ± 9.86	25.24 ± 7.87	63.64 ± 11.31	23.96 ± 4.14
72	25.19 ± 8.50	7.08 ± 3.82	30.40 ± 5.50	9.15 ± 4.85
120	11.67 ± 5.99	<LOQ	14.45 ± 4.09	ND[b]
168	6.49 ± 2.35	ND[b]	5.39 ± 2.53	ND[b]
216	<LOQ	ND[b]	<LOQ	ND[b]

[a]Each value represents the mean ± SD for five pigs. [b]Not detected.

Table 4. Average Concentrations of Cyx, Cy1, Cy2, and QCA in Fat after Multiple Oral Administrations of Cyx at a Dose of 20 mg/kg of Body Weight for Five Consecutive Days

	concentration[a] (ng/g)			
time (h)	Cyx	Cy1	Cy2	QCA
12	48.80 ± 11.47	73.18 ± 23.43	89.02 ± 23.86	26.06 ± 5.61
24	25.50 ± 5.12	28.44 ± 12.63	33.94 ± 9.48	11.54 ± 2.50
72	13.32 ± 2.86	7.31 ± 2.48	13.66 ± 3.06	5.64 ± 0.78
120	6.43 ± 3.42	<LOQ	5.72 ± 0.85	ND[b]
168	<LOQ	ND[b]	ND[b]	ND[b]
216	ND[b]	ND[b]	ND[b]	ND[b]

[a]Each value represents the mean ± SD for five pigs. [b]Not detected.

Interestingly, in this study, when pigs were orally treated with Cyx at a dose of 20 mg/kg of body weight for five consecutive days, the concentration of Cyx in muscle initially increased and then decreased slowly. This phenomenon was not observed in other tissues (liver, kidney, and fat). It is suggested that Cyx reached the maximal concentration in liver, kidney, and fat more quickly than in muscle. The times that Cyx reached the maximal concentration in muscle, fat, liver, and kidney were 24, 12, 12, and 12 h after treatment, respectively.

Cyx was undetectable in the liver and kidney of pigs 120 h after the last administration; the concentrations of Cyx in muscle and fat decreased to below the LOD 216 and 168 h after withdrawal, respectively. The order of the rates of elimination of Cyx in pigs was as follows: kidney > liver > fat > muscle. Huang et al. reported that when six chickens were treated with Cyx at a clinical recommended dose of 100 mg/kg of body weight (in feed) for 10 consecutive days, only small amounts of Cyx were found in muscle, fat, liver, and kidney 0.5 h after administration[14] and Cyx could not be detected in muscle and fat at 2 h; however, the Cyx parent drug was still detectable in liver and kidney 6 h after administration.[23]

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Table 5. Average Concentrations of Cyx, Cy1, Cy2, and QCA in Liver after Multiple Oral Administrations of Cyx at a Dose of 20 mg/kg of Body Weight for Five Consecutive Days

	concentration[a] (ng/g)			
time (h)	Cyx	Cy1	Cy2	QCA
12	27.80 ± 7.96	168.36 ± 64.51	70.18 ± 27.06	256.78 ± 72.80
24	14.80 ± 4.63	63.44 ± 24.46	25.56 ± 14.94	124.38 ± 33.41
72	5.64 ± 2.64	25.20 ± 9.64	6.67 ± 3.62	68.86 ± 27.84
120	ND[b]	6.82 ± 3.87	<LOQ	22.12 ± 13.37
168	ND[b]	<LOQ	ND[b]	7.45 ± 3.98
216	ND[b]	ND[b]	ND[b]	<LOQ

[a]Each value represents the mean ± SD for five pigs. [b]Not detected.

Table 6. Average Concentrations of Cyx, Cy1, Cy2, and QCA in Kidney after Multiple Oral Administrations of Cyx at a Dose of 20 mg/kg of Body Weight for Five Consecutive Days

	concentration[a] (ng/g)			
time (h)	Cyx	Cy1	Cy2	QCA
12	34.64 ± 16.12	374.28 ± 184.87	61.12 ± 22.27	537.52 ± 266.80
24	19.08 ± 8.92	173.32 ± 123.31	23.96 ± 12.10	334.34 ± 130.43
72	6.41 ± 3.85	76.68 ± 36.78	6.61 ± 2.66	155.06 ± 100.66
120	ND[b]	25.62 ± 12.50	ND[b]	60.32 ± 22.81
168	ND[b]	6.26 ± 3.49	ND[b]	26.38 ± 15.90
216	ND[b]	ND[b]	ND[b]	7.68 ± 3.09

[a]Each value represents the mean ± SD for five pigs. [b]Not detected.

Table 7. Main Pharmacokinetic Parameters of Cyx, Cy1, Cy2, and QCA in Liver, Kidney, Muscle, and Fat in Treated Pigs after Multiple Oral Administration of Cyx at a Dose of 20 mg/kg of Body Weight for Five Consecutive Days

compound	tissue	AUC[a] (h ng g^{-1})	β[b] (h^{-1})	$t_{1/2\beta}$[c] (h)	MRT[d] (h)
Cyx	liver	1141.38	0.025	28.04	43.88
	kidney	1383.10	0.026	26.08	40.66
	muscle	4482.11	0.014	49.05	75.25
	fat	2591.75	0.015	48.28	67.45
Cy1	liver	5590.71	0.023	29.84	40.17
	kidney	15351.15	0.026	26.55	44.13
	muscle	1958.10	0.034	20.00	31.11
	fat	2112.78	0.036	19.52	30.42
Cy2	liver	1954.51	0.036	19.25	29.95
	kidney	1804.74	0.034	20.31	31.64
	muscle	5861.02	0.017	40.83	58.72
	fat	3188.00	0.019	37.38	48.50
QCA	liver	11679.86	0.021	31.91	50.50
	kidney	28657.33	0.020	35.02	54.35
	muscle	1960.72	0.028	25.09	39.38
	fat	1059.80	0.023	29.68	48.84

[a]Area under the concentration−time curve from 0 h to ∞. [b]Elimination rate constant. [c]Elimination half-life. [d]Mean residue time.

In fact, metabolite Cy1 concerns much of our study. Studies have shown that the N → O group contributed to the carcinogenic, teratogenic, and mutagenic activities of quinolone derivatives.[24−26] The N → O group can produce some -OH free radicals and toxic intermediate free radicals in the process of deoxygenation, which can bind with DNA or protein and induce toxicity, resulting in broken DNA or protein denaturation.[27]

Cy2 is a Cyx N → O group reduction metabolite. A previous report has shown that the major metabolic pathway of Cyx is N → O group reduction and hydrolysis of the amide bond, followed by hydroxylation and reduction of the double bond.[8] This implied that Cy2 was further metabolized to Cy1; therefore, the amount of Cy2 was not large in various tissues. In comparative terms, the concentrations of Cy2 were high in muscle and fat. Its residual elimination time was shorter than that of Cyx in swine tissues, and the elimination half-lives of Cy2 were 40.83, 37.38, 19.25, and 20.31 h in muscle, fat, liver, and kidney, respectively. Cy2 was eliminated quickly in the liver and kidney of swine. In our research, the concentrations of Cy1 were relatively high in liver and kidney, with maximal concentrations of 168.36 ± 28.85 and 374.28 ± 82.68 ng/g, respectively. This result was not consistent with the fact that Cy2 was abundant in swine intestinal microsomes.[9] A different distribution of enzymes in liver and kidney tissues as well as a different metabolism *in vivo* and *in vitro* may account for this.[8]

In the study presented here, when pigs were orally treated with Cyx at a dose of 20 mg/kg of body weight for five consecutive days, QCA was not detected in muscle or fat 120 h after drug withdrawal and the concentrations of QCA in liver and kidney decreased to below the LOD (2 ng/g) 9 and 11 days after withdrawal, respectively. The highest concentrations of QCA in liver and kidney were 256.78 ± 32.56 and 537.52 ± 119.32 ng/g, respectively. Until now, the residue marker and target tissue for Cyx have not been identified. To guarantee human health and reduce potential hazards, on the basis of mass reasons, it was proposed that QCA might potentially be the residue marker of Cyx and kidney might be the target tissue in pigs.

In summary, we, for the first time, have investigated the deposition and elimination of Cyx as well as its three major metabolites in pigs after multiple oral administrations at a dose of 20 mg/kg of body weight for five consecutive days. The results of this research, on one hand, confirm the validity of the method developed by Yan et al.;[16] on the other hand, the results provide a comprehensive understanding of the metabolism and distribution of Cyx and its three major metabolites in swine tissues.

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Y.L. and N.Z. contributed equally to this work.

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Notes

The authors declare no competing financial interest.

ABBREVIATIONS USED

Cyx, cyadox; OLA, olaquindox; CBX, carbadox; Cy1, 1,4-bisdesoxycyadox; Cy2, 4-desoxycyadox; QCA, quinoxaline-2-carboxylic acid; MeOH, methanol; ACN, acetonitrile; FC, formic acid; DMSO, dimethyl sulfoxide; HPLC−MS/MS, high-performance liquid chromatography−tandem mass spectrometry; EA, ethyl acetate; CF, chloroform; MRM, multiple-reaction monitoring; LOD, limit of detection; LOQ, limit of quantification; CV, coefficient of variation; AUC, area under the concentration−time curve; $t_{1/2\beta}$, depletion half-life; β, elimination rate constant; MRT, mean residue time; SD, standard deviation

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