Pharmacokinetics of Oxytetracycline in the High Economic Valuable Chinese Soft-Shell Turtle (*Pelodiscus sinensis*)

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Received: November 8th, 2020.

Accepted: November 18th, 2020.

Published Online: November 19th, 2020.

Journal: International Journal of Veterinary Research

Publisher: Medcare Science Publisher.


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Keywords: Chinese Soft-Shell Turtle; Drug Residue; High-Performance Liquid Chromatography; Oxytetracycline; Pharmacokinetics.

Abstract

The aim of this study was to understand the pharmacokinetics of ox tetracycline (OTC) in the Chinese soft-shell turtle, Pelodiscus sinensis by using HPLC-UV detection. Turtles were treated with OTC at a medical concentration of 30 mg/kg body weight once by gavage. These results showed that the OTC recovery rates in the liver, muscle, and sera of turtles were 70–87%, 98–103%, and 88–98%, respectively. After OTC administration, the estimated AUC0-inf in the serum was 1,443.00 ± 92.30 h × μg/mL. The half-life (t/2) of OTC was estimated to be 29.50 ± 0.22 h. The mean residence time MRT was 40.00 ± 10.20 h. The observed Tmax occurred at 4.00 ± 0 h and the maximum serum concentration (Cmax) was the highest at 2.17 ± 0.32 μg/mL for 4 h. OTC concentration in the serum was below the limit of detection (LOD) (< 0.05 μg/mL) on day 8 after p.o. with OTC. The average drug residue concentrations of OTC in the liver and muscle were the highest on day 1 administration. Residual concentrations of OTC in the liver and muscle on day 24 administration were below LOD. This information of Chinese soft-shell turtles will provide greater protection to consumers and design rational OTC residue dosing regimens in formulating laws and regulations in Taiwan government.

Introduction

There are many species of tortoise and turtles on Earth. Among them, the turtle is the most ancient reptile (Shine, 2013) and consists of numerous species (Xu et al., 2018). High commercial values of turtles were severed as a source of medicine, healthy food, and as aqua-pets. Thus, they are subjected to extensive harvest (Li et al., 2017). The Chinese soft-shell turtle (Pelodiscus sinensis) reported that widely distributed freshwater in China and its annual output reached 340,000 tons in 2014 (Bureau of Fisheries, Ministry of Agriculture, China, 2015). It is also distributed in Asian countries as Korea, Japan, and Vietnam, etc. (Fritz et al., 2010; Li et al., 2016). In the Fisheries Statistics Annual Report of Taiwan, the annual output reached 190,000, 205,000, 250,000, 210,000, and 180,000 tons in 2000, 2005, 2010, 2015, and 2017, respectively. According to the statistics of the Taiwan Turtle Aquaculture Association, Chinese soft-shell turtles are sold as two types: mature turtles and turtle eggs. Approximately 200 million turtle eggs are exported each year and mature turtles are mainly sold domestically.

One of the most common turtle species is Chinese soft-shell turtle widely cultured and frequently applied in Taiwan (Zhang et al., 2018). Recently, Chinese soft-shell turtles became economically important and are produced at a large-scale in East Asian countries, such as China, Japan, Korea, and Taiwan. The turtles exhibit more rapid growth and lower fat levels (Sun et al., 2017; Zhang et al., 2018). In addition, turtle eggs are also recognized as a healthy food (Yu et al., 2005; Shen et al., 2008), which have a cholesterol-lowering effect and can prevent hypertension (Yu et al., 2005; Rawendra et al., 2013; Chen and Kuo, 2016). The Chinese soft-shell turtle and its eggs have high nutritional and medicinal values; therefore, Chinese soft-shell turtle is a commercially important cultured species. Under large-scale and high-density culture, many diseases were found in the commercial turtle farms. In China, Chinese soft-shell turtle is vulnerable infection with Paecilomyces lilacinus, Aeromonas spp., iridovirus, and Bacillus thuringiensis (Chen et al., 1999; Zhou et al., 1999; Chen et al., 2013; Chen et al., 2014; Shi et al., 2018). Additionally, poxivirus infection caused vesicular diseases in commercially cultured Chinese soft-shell turtles has been reported in 2011 in Taiwan (Liu et al., 2011).

OTC (oxytetracycline) is one of the often applied antibiotics in aquaculture disease control and treatments (Rigos and Smith, 2015). It is a broad-spectrum antibiotic that belongs to the tetracycline group and has been widely used in aquaculture disease treatments frequently administered through the feed or in bath (immersion therapy) of aqua-pathogens in fish, such as furunculosis, aeromonos, pseudomonos, lactococcosis, and vibriosis (AliAbadi et al., 2002; Gastalho et al., 2014; Cenavisa, 2016; Drugs.com, 2016; Leal et al., 2016; Leal et al., 2017). The main target fish species for OTC treatments are salmonids: Salmo salar and Oncorhynchus mykiss, Icterus punctatus, Oreochromis niloticus, Paralichthys olivaceus, Scophthalmus maximus, Diplodus sargus, L., Dicentrarchus labrax L., Anguilla anguilla L., and Cyprinus carpio L. (Seckin and Kum, 2011; Cenavisa, 2016; Leal et al., 2018). Based on pharmacological research on OTC, the mode of bactericidal action is the establishment of strong complexes with Ca2+ and Mg2+ ions, thereby inhibiting protein synthesis, which may have implications on biological activity or poor metabolism by fish (Leal et al., 2018). Laboratory studies concerning the pharmacokinetics of OTC in products of Chinese soft-shell turtles are very limited. However, the pharmacokinetics of OTC in aquaculture have been studied in many aquatic species, including blunt-snout bream.
Litopenaeus vannamei (Li et al., 2015), farmed Pacific white shrimp (Litopenaeus vannamei) (Chiayavaresaja et al., 2006), cultured tilapia (Paschoal et al., 2012), sea bass [Dicentrarchus labrax (L.)] (Rigos et al., 2004), giant freshwater prawns (Macrobrachium rosenbergii) (Poapolathep et al., 2008b), Chinese mitten crabs (Eriocheir sinensis) (Feng et al., 2010), farmed Atlantic salmon (Brocklebank et al., 1997), channel catfish (O’Hara et al., 1997), and giant freshwater prawns (Macrobrachium rosenbergii) (Brillantes et al., 2001). Only one study exists, which addressed the pharmacokinetics of OTC in an aquatic turtle, specifically the loggerhead sea turtle (Caretta caretta) (Harms et al., 2004). Moreover, the pharmacokinetic profiles of OTC have not been published for freshwater turtles until now.

Currently, the Chinese soft-shell turtle culture industry is important in Taiwan and China. Thus, the use of antibiotics in Chinese soft-shell turtle culture has become very important. To date, there have been very few reports on the pharmacokinetics of OTC in freshwater and seawater turtles. Therefore, the goals of this study were to evaluate the pharmacokinetics of OTC after oral administration (p.o.) in the freshwater turtles. Pharmacokinetic investigations of antibiotics are important for defining the dosage regimens (Ang et al., 1996). The edible aquaculture tissues with OTC residues could be affected the consumers’ healthy (Luo and Ang, 2000). Thus, a proper withdrawal period for OTC is needed to ensure the edible tissues are safe for the consumers (Hung et al., 2018; Hung et al., 2019). Consequently, the aim of the research is to determine the OTC pharmacokinetic parameter values in Chinese soft-shell turtles via p.o. and using high-performance liquid chromatography (HPLC) with ultraviolet (UV) detection. This rapid, simple, and selective method is suitable for pharmacokinetics, tissue distribution, and depletion of OTC in Chinese soft-shell turtles after p.o. of OTC single-dose via medicated feed.

In addition, the maximum OTC residue level is 0.1 mg/kg in muscles and skin of Chinese soft-shell turtles according to Standards for Veterinary Drug Residues in Aquatic Animal Drugs Use in Taiwan. Because there are fewer reports about the pharmacokinetic studies of Chinese soft-shell turtles and the withdrawal period of OTC is unknown, therefore, a rapid and reliable technique for evaluating the pharmacokinetics of OTC is needed. We hope this research will provide some basic information about formulating effective and reasonable OTC use regimens in freshwater turtles.

**MATERIALS AND METHODS**

**Preparation of chemicals**

In this study, acetonitrile and methanol (HPLC-grade) were ordered from Aencore Chemical (Surrey Hills, Australia). n-Hexane, citric acid, Sodium dihydrogen phosphate monohydrate, and Disodium ethylene di amine tetra acetate were ordered from Merck (Kenilworth, New Jersey, USA). Formic acid, trichloroacetic acid, and oxytetracycline (OTC; C_{22}H_{24}N_{2}O_{8}; Figure 1A) were ordered from Sigma-Aldrich (St. Louis, MO, USA).

**Preparation of the standard solution and the extract solutions**

Preparation of the OTC standard solution was conducted according to previously described procedures (Hung et al., 2018; Hung et al., 2019).

**HPLC apparatus**

The HPLC system and analysis of HPLC data were carried out according to previously described procedures (Hung et al., 2018; Hung et al., 2019). An ultraviolet (UV) detector with 270 nm detection wavelength was applied.

**Validation method**

The mean duration of estrus in zebu cattle is around 10 hours, but with a range of 1.3 to 20.0 hours (Table 1). Estimates of the duration of estrus in Bos taurus cattle in the tropics and subtropics range from about 11 hours to about 15 hours. The wide variation in the duration of estrus in zebu cattle partly reflects variation in the observation methods and actual breed peculiarities of estrus in zebu cattle, some of which are highlighted below [12, 23].

Linearity was carried out according to previously described procedures (Hung et al., 2018; Hung et al., 2019). 0.01, 0.05, 0.2, 0.5, 0.8, and 1 μg/mL OTC were respectively supplemented into the blank sera, blank muscles, and liver. After residue reconstitution with 0.1% formic acid-acetonitrile (90 : 10, v/v; pH 3), the internal standard was finally added. The maximum absorption wavelength and flow rate was 270 nm and 1 mL/min, respectively.

**Quantification**

Standardization and construction of calibration curves were carried out according to previously described procedures (Hung et al., 2018; Hung et al., 2019). Samples of 1 mL blank sera, 2 g blank muscle, and 2 g blank liver were respectively supplemented with known amounts of OTC and the internal standard and were analyzed as unknown samples.

**Accuracy**

Accuracy was carried out according to previously described procedures (Hung et al., 2018; Hung et al., 2019). The blank tissues as muscle, sera, and liver were respectively supplemented with 10, 1, and 0.1 μg/mL OTC and the internal standard were analyzed repeatedly.

**Limits of quantification and detection (LOQ and LOD) and specificity**

LOD, LOQ, and specificity of OTC were carried out according to previously describe procedures (Hung et al., 2018; Hung et al., 2019).

**Validation of intra-assay and inter-assay**

The assay validation of OTC was conducted according to described previously (Ramos et al., 2003; Hung et al., 2019). The concentration of OTC (10, 1, and 0.1 μg/mL) in samples was used for the determination of the linear regression and the precision of the method.

**Animal care and the experimental setup**

Animal care in this study were carried out according to previously described the guidelines of the Institutional Animal Care and Utilization Committee (IACUC). Male Chinese soft-shell turtles (Pelodiscus sinensis) (total of No. is 170) were fed a standard laboratory diet twice a day. Environment conditions were kept on a 12 h dark/light cycle and temperature was set at 20 ± 2°C and water pH was set at 6.8 ± 0.4. Healthy male Chinese soft-shell turtles (500 ± 10 g) were obtained from aecommercial Chinese soft-shell turtle farm (Neipu Township, Pingtung County, Taiwan) and were housed in a 300 L tank (length × width × depth is 120 cm × 60 cm × 45 cm) with a
freshwater recirculation system. Experiments began after an acclimation period of 2 weeks. Before the experiment, liver, sera, and muscle were collected from 6 Chinese soft-shell turtles to confirm OTC residues in these samples.

Sample collection of Chinese soft-shell turtles for OTC residual tests

First, sera were collected from 6 Chinese soft-shell turtles per time point (1, 2, 4, and 8 hour, and 1, 2, 4, 8, 12, 16, 20, and 24 day) after single p.o. of OTC (30 mg/kg BW; the medical dose). Additionally, these collected sera were stocked at -20°C.

Sample collection and process of Chinese soft-shell turtles for pharmacokinetic analysis of OTC

Six Chinese soft-shell turtles per group were given single p.o. of OTC (30 mg/kg BW), whereas age and size matched 6 control Chinese soft-shell turtles were p.o. of an equal volume of normal saline. Approximately 0.5 mL blood, muscle, and liver samples were collected from each Chinese soft-shell turtles on 1, 2, 4, and 8 hour, and 1, 2, 4, 8, 12, 16, 20, and 24 day after OTC administration. Additionally, the consecutive progress of blood samples, muscle, and liver were carried out according to previously described procedures (Hung et al., 2018; Hung et al., 2019).

Sample clean-up, derivatization, and extraction

The sample clean-up, derivatization, and extraction were conducted according to previously described procedures (Ramos et al., 2003; Hung et al., 2018; Hung et al., 254, 2019).

Pharmacokinetic data analysis

Pharmacokinetic data analysis were carried out according to previously described (Hung et al., 2018; Hung et al., 2019). Pharmacokinetic data analysis of OTC in sera of Chinese soft-shell turtle (n = 6 / the experimental point) was using model-independent standard methods. The parameters evaluated for serum were Tmax (time to maximum concentration, h), t1/2 (half-life, h), Cmax (maximum concentration, μg/mL), MRT (mean residence time, h), and AUC0-inf (area under the concentration-time curve from 0 h to infinity, h x mg/L).

Statistical analysis

Statistical analysis were carried out according to previously described (Hung et al., 2018; Hung et al., 2019). The statistical significant difference was at P < 0.05 by using Student’s t test.

RESULTS

The standard curve at various OTC concentrations

The results showed an obvious peak for 10 μg/mL OTC at 15.933 min under the UV detector (the maximum absorption of the OTC standard was at 270 nm) (Figure 1B). The standard curve at various OTC concentrations (0.01, 0.05, 0.1, 0.5, and 1 μg/g) was Y = 2.1937 × 10^5X + 2.1441 (X: concentration; Y: area), r² = 0.9994 (Figure 2). LOD and LOQ were, respectively, 1 and 5 ng/mL. An r² value of calibration curve is 0.9994. The coefficient of variation (C.V.) of intra-day precision was 0.61–4.37% (mean C.V. is 2.14%) (Table 1). The C.V. of inter-day precision was 0.64–4.59% (mean C.V. is 1.62%) (Table 1). The C.V. of precision was lower than the 10% that indicated the precision was acceptable in this study. HPLC chromatographs of various OTC concentrations (0.05, 0.1, 0.2, 0.5, 0.8, and 1 μg/mL) were showed nearing 16 minutes in the spiked tissues compared to the spiked blank tissues. The standard curve was Y = 2.1281 × 10^5X – 769.58 and the r² value was 0.9977 for the spiked sera with OTC (Figure 3A). The mean recovery (%) was 88–98%. LOD of OTC in sera was 0.05μg/mL. The standard curve was Y = 2.11151× 10^-X – 154.99 and the r² value was 0.9989 in the spiked muscle with OTC (Figure 3B). The mean recovery (%) was 98–103%. LOD of OTC in muscle was 0.02 μg/mL.

The standard curve was Y = 2.0757 × 10^-X – 392.93 and the r² value was 0.9992 in the spiked liver with OTC (Figure 3C). The mean recovery (%) was 70–87%. LOD of OTC in liver was 0.05 μg/mL.
OTC concentrations in Chinese soft-shell turtle tissues p.o. with 30 mg/kg BW at various time points

OTC concentrations of Chinese soft-shell turtle muscle and liver were followed p.o. with 30 mg/kg BW at various time points (hour 1, 2, 4, and 8, and day 1, 2, 4, 8, 12, 16, 20, and 24) of the experiment are shown in Figures 4-5. At 4 h after OTC p.o. to Chinese soft-shell turtles, OTC concentration in sera was the highest (2 μg/mL). After the 4 h OTC p.o, the OTC concentration in sera decreased gradually. At 24 h and 96 h after OTC p.o., OTC concentration in the sera was 0.46 and 0.12 μg/mL, respectively. At 192 h (8 days) after OTC p.o., OTC concentration in sera was below the LOD (0.05 μg/mL). On the other tissues, OTC concentrations detected in the liver and muscle following p.o. 30 mg/kg BW OTC at 1, 2, 4, 8, 12, and 24 days. On day 1, 8, and 24 post 30 mg/kg BW OTC p.o., the mean muscular OTC concentration was 1.28, 0.2, and < 0.02 (LOD) μg/mL, respectively. On day 1, 8, and 24 post 30 mg/kg BW OTC p.o., the mean OTC concentration in the liver was 1.47, 0.36, and < 0.05 (LOD) μg/mL, respectively (Table 2).

Table 1: Accuracy and precision for OTC determination using HPLC with a UV detector (n = 3 per treatment).

<table>
<thead>
<tr>
<th>Day</th>
<th>Intra/inter-day</th>
<th>OTC (μg/mL)</th>
<th>Area (mean)</th>
<th>S.D.</th>
<th>C.V. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st</td>
<td>Intra-day</td>
<td>0.1</td>
<td>2.584</td>
<td>113</td>
<td>4.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>22.909</td>
<td>140</td>
<td>0.61</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>260.793</td>
<td>3,760</td>
<td>4.37</td>
</tr>
<tr>
<td>1st</td>
<td>Inter-day</td>
<td>0.1</td>
<td>2.462</td>
<td>113</td>
<td>4.59</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>23.703</td>
<td>400</td>
<td>1.69</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>266.851</td>
<td>3,896</td>
<td>1.46</td>
</tr>
<tr>
<td>2nd</td>
<td></td>
<td>0.1</td>
<td>2.280</td>
<td>50</td>
<td>2.20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>21.089</td>
<td>233</td>
<td>1.10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>277.533</td>
<td>1,784</td>
<td>0.64</td>
</tr>
<tr>
<td>3rd</td>
<td></td>
<td>0.1</td>
<td>2.503</td>
<td>22</td>
<td>0.88</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>22.091</td>
<td>158</td>
<td>0.72</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>254.310</td>
<td>3,397</td>
<td>1.34</td>
</tr>
</tbody>
</table>

Figure 3: Standard curve of OTC in the spiked tissues of Chinese soft-shell turtles. (A) In the spiked sera, the standard curve is Y = 2.1281 × 10^4X + 769.58 and r^2 value is 0.9977. (B) In the spiked muscle, the standard curve is Y = 2.1115 × 10^4X – 154.99 and r^2 value is 0.9989. (C) In the spiked liver of Chinese soft-shell turtles, the standard curve is Y = 2.0757 × 10^4X – 392.93 and r^2 value is 0.9992. X: concentration; Y: area.
**Table 2:** OTC residue concentrations in the liver and muscle of Chinese soft-shell turtles (n = 6 per groups) at various time points during the experiment.

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Liver (ppm)</th>
<th>Muscle (ppm)</th>
<th>Blank (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.62 ± 0.08</td>
<td>0.48 ± 0.03</td>
<td>–</td>
</tr>
<tr>
<td>2</td>
<td>0.39 ± 0.04</td>
<td>0.47 ± 0.30</td>
<td>–</td>
</tr>
<tr>
<td>4</td>
<td>0.77 ± 0.23</td>
<td>0.45 ± 0.01</td>
<td>–</td>
</tr>
<tr>
<td>8</td>
<td>0.46 ± 0.02</td>
<td>0.41 ± 0.06</td>
<td>–</td>
</tr>
<tr>
<td>12</td>
<td>1.13 ± 0.80</td>
<td>0.56 ± 0.50</td>
<td>–</td>
</tr>
<tr>
<td>24</td>
<td>1.47 ± 0.10</td>
<td>1.28 ± 0.06</td>
<td>–</td>
</tr>
<tr>
<td>48</td>
<td>0.53 ± 0.15</td>
<td>0.48 ± 0.08</td>
<td>–</td>
</tr>
<tr>
<td>96</td>
<td>0.51 ± 0.07</td>
<td>0.49 ± 0.06</td>
<td>–</td>
</tr>
<tr>
<td>192</td>
<td>0.36 ± 0.09</td>
<td>0.20 ± 0.09</td>
<td>–</td>
</tr>
<tr>
<td>288</td>
<td>0.15 ± 0.03</td>
<td>0.20 ± 0.07</td>
<td>–</td>
</tr>
<tr>
<td>384</td>
<td>0.09 ± 0.02</td>
<td>0.16 ± 0.03</td>
<td>–</td>
</tr>
<tr>
<td>480</td>
<td>0.09 ± 0.02</td>
<td>0.08 ± 0.02</td>
<td>–</td>
</tr>
<tr>
<td>576</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

‘–’ : lower than the LOD.

**Pharmacokinetic analysis of OTC in Chinese soft-shell turtles**

Detection of OTC concentration in sera post p.o. with 30 mg/kg BW at 1, 2, 4, 8, 12, and 24 h(s). At each time point, 6 Chinese soft-shell turtles were used. The pharmacokinetic non-compartmental parameters estimated after p.o. with 30 mg/kg BW OTC to Chinese soft-shell turtles (Table 3). After p.o. with OTC, AUC0-inf in the sera was 1,443.00 ± 92.30 h × μg/mL; t1/2 was 29.50 ± 0.22 h; MRT was 40.00 ± 10.20 h; Tmax occurred at 4.00 ± 0 h; Cmax was the highest at 2.17 ± 0.32 μg/mL p.o. with OTC for 4 h. OTC concentration in sera was below LOD on day 8 after p.o. with OTC.

**DISCUSSION**

The freshwater Chinese soft-shell turtle is distributed widely in China (distributed from Hainan to Inner Mongolia) (Shi et al., 2019). It is an important aquaculture species in China (Bureau of Fisheries, Ministry of Agriculture, China, 2015). It is also wildly distributed in Taiwan and other Asian countries (Fritz et al., 2010; Li et al., 2016). The Chinese soft-shell turtle is one of the most important turtles, having a high economic value in the food industry in Taiwan and China. Recently, water pollution and environmental stress caused bacterial infections of the freshwater Chinese soft-shell turtle as “red neck diseases” induced by pathogenic Aeromonas and “putrid-skin diseases” induced by pathogenic Pseudomonas and Aeromonas. Bacterial infections of the freshwater Chinese soft-shell turtle have led to serious mass mortalities (Chen et al., 1999; Zhou et al., 1999; Orós et al., 2003; Chen et al., 2013; Chung et al., 2017; Shi et al., 2019). OTC is an antimicrobial that has been used to treat or prevent bacterial infections (exhibiting activity against a wide range of Gram-positive and Gram-negative bacteria).
range of bacteria) for many years and has wide application, including human, veterinary, and agricultural uses throughout the world (Leal et al., 2016). OTC has been widely used for treatment in fish aquaculture to prevent or treat aquatic-pathogen infections (AliAbadi, 2002; Rigos and Smith, 2015; Cenavisa, 2016; Drugs.com, 2016; Leal et al., 2018). In Taiwan, 14 antibiotics are allowed for use in aquaculture. Among them, there are 5 antibiotics (OTC, sulphonamethoxine, oxolinic acid, flumequine, and florfenicol) allowed for use in the order Testudines. According to Taiwan laws, OTC can be used at 50 mg/kg per day continuously for 3–5 days to treat infections caused by Aeromonas hydrophilia or Pseudomonas fluorescens. Antibacterial agents are necessary for Chinese soft-shell turtle cultures to cope with emerging bacterial diseases (Chung et al., 2017). OTC were frequently applied in aquatic animal disease control and treatments and numerous OTC pharmacokinetic researches have been conducted (Chung et al., 2017; Shi et al., 2019). In this study, we firstly reported the OTC pharmacokinetic characteristics in Chinese soft-shell turtles post p.o. with a single-dose of 30 mg/kg BW OTC (the medical dose). Thirty milligrams per kilogram BW for the medical dose was selected, modified, and referred (Gould, 1998). In Taiwan government, the recommended maximum OTC residue level in the muscle and skin of the order Testudines is 0.1 mg/kg (ppm) according to the Standards of Veterinary Drug Residues in Aquatic Animal Drugs Use. Many articles were published to study the pharmacokinetics of OTC in aquaculture of fish (Uno et al., 1992; Abedini et al., 1998; Rigos et al., 2003; Grondel et al., 2010; Rigos et al., 2010), crustaceans (shrimp and prawn) (Brillantes et al., 2001; Reed et al., 2004; Sangrurunguang et al., 2004; Uno, 2004; Chiayvareesajja et al., 2006; Uno et al., 2006; Faroongsarng et al., 2007; Gómez-Jimenez et al., 2008; Poapolathep et al., 2008a,b; Uno et al., 2010; Uno et al., 2016), and sea turtles (Harms et al., 2004). OTC pharmacokinetics in *Megalobrama amblycephala* with single and multiple-dose p.o. was investigated. For the single dose of OTC p.o., the results showed that t1/2 was 5.79, 9.40, 6.96, and 8.06 h in the plasma, liver, kidney, and muscle, respectively (Li et al., 2015). In addition, an OTC residue depletion research in the farmed Atlantic salmon to assess the physiologically based pharmacokinetic model was conducted (Brocklebank et al., 1997). Others reported the pharmacokinetic analysis of OTC residues in Chinese mitten crab muscle post intramuscular administration with respective 2, 8, and 40 μg/kg BW OTC (Feng et al., 2010) and the complete OTC pharmacokinetic analysis for the farmed Pacific white shrimp (Chiayvareesajja et al., 2006). Currently, there is one study on the OTC pharmacokinetics in a sea turtle (Harms et al., 2004). The pharmacokinetics of OTC (25 mg/kg BW) after single intravenous and intramuscular administrations in loggerhead sea turtles was investigated. OTC concentrations in plasma were analyzed by reverse-phase HPLC. For the intravenous route of OTC, means for *Cmax*, t1/2, CI (systemic clearance), and Vdss (apparent volume of distribution at steady state) were 6.6 μg/mL, 66.1 h, 290.7 mL/h x kg, and 18.4 L, respectively. For the intramuscular route of OTC, means for systemic availability, *Cmax*, and t1/2 were 91.8%, 1.6 μg/mL, and 61.9 h, respectively. The current study is the first to report the OTC pharmacokinetics in a freshwater turtle via p.o. by gavage. The AUC0-inf in sera, t1/2, MRT, *Tmax*, and *Cmax* were 1,443.00 ± 92.30 h x μg/mL, 29.50 ± 0.22 h, 40.00 ± 10.20 h, 4.00 ± 0 h, and 2.17 ± 0.32 μg/mL, respectively. Although the OTC administration routes were different in the seawater and freshwater turtles, we also compared the same pharmacokinetic reference indexes as *Cmax* and t1/2 in the seawater and freshwater turtles. *Cmax* in the freshwater turtles via intravenous and intramuscular administration was, respectively, 6.6 and 1.6 μg/mL; *Cmax* in the freshwater turtles via p.o. was 2.17 ± 0.23 μg/mL. t1/2 in the seawater turtles via intravenous and intramuscular administration was, respectively, 66.1 and 61.9 h; t1/2 in the freshwater turtles via p.o. administration was 29.50 ± 0.22 h. Taken together, some factors, such as different administration routes of OTC, environment salinity, and turtle species, could have affected OTC pharmacokinetics. At present, very few OTC pharmacokinetic studies in turtles have been published. Therefore, additional information of OTC pharmacokinetics in turtles, especially freshwater turtles, is very important. Although maximum plasma concentration by intramuscular administration was lower than that of the intravenous route in the sea turtle, the long elimination time indicated that an infrequent dosing interval may be effective for bacteria (Harms et al., 2004). In addition, the mean OTC residue concentrations in the liver and muscle on day 24 after the 30 mg/kg BW OTC single p.o. were below the LOD (0.05 and 0.02 μg/mL in this study). Thus, we recommend 24 days was a withdrawal period after OTC administration in Chinese soft-shell turtles. According to the literature, OTC can be analyzed by using many methods as thin-layer chromatography, HPLC, capillary electrophoresis, spectrophotometry, chemiluminescence detection, microbiological assays, and flow injection analysis.

However, HPLC is the most widely used and recommended by official compendia. Unfortunately, the development of a reliable, simple, and rapid technique to detect the OTC pharmacokinetics and its residual levels in Chinese soft-shell turtles has been lacking. In this study, our data presented that the OTC retention time was 15.933 mins with an OTC LOD and LOQ of 1 ng/mL and 5 ng/mL, respectively, and that tissue recoveries range between 88%–98% in sera, 98%–103% in muscle, and 70%–87% in liver. Herein, solid-phase extraction (SPE) and HPLC with UV detector can produce satisfactory results for OTC pharmacokinetics in Chinese soft-shell turtles’ muscle, liver, and sera.

The ensuing risks of the drug residues in the edible products have become a public safety issue. According to Taiwan standard of drug residues, the maximum legally allowed residue limits in the muscle and skin of turtles is 0.1 μg/mL (0.1 ppm). Based on our results in this study, OTC concentrations in the muscle and liver on day 24 post p.o. with 30 mg/kg BW OTC were both lower than LOD (0.02 ppm in muscle and 0.05 ppm in liver). Additionally, OTC was rapidly absorbed and eliminated in Chinese soft-shell turtles with a serum *Tmax* of 4.00 ± 0 h, a *Cmax* of 2.17 ± 0.32 μg/mL, a t1/2 of 29.50 ± 0.22 h, and an MRT of 40.00 ± 10.20 h in the present study. Taken all information together, the maximum residue limit in muscle and liver of Chinese soft-shell turtles was below the legally allowed 0.1 ppm after 480 h- and 384 h- post a single dose p.o. with 30 mg/kg BW OTC, respectively. Thus, we recommend 24 days is an OTC withdrawal period after OTC administration in Chinese soft-shell turtles.

**CONCLUSION**

An accurate, precise, and quite sensitive HPLC-UV method for OTC determination in Chinese soft-shell turtles was developed. This is the first report on pharmacokinetic study of OTC for Chinese soft-shell turtles. The pharmacokinetic information of OTC in Chinese soft-shell turtles could be provide to Taiwan government to design rational dosing regimens and formulate laws and regulations for determining permissible OTC residue limits. This information could be also provided greater protection to consumers.
ACKNOWLEDGEMENTS

The authors thank the Council of Agriculture in Taiwan (Executive Yuan) for supporting this study.

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