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Pharmacokinetics and bioavailability of oxolinic acid in vannamei shrimp (*Penaeus vannamei*) and the effect of processing on the residues

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Abstract

The present study examined the pharmacokinetics and bioavailability of oxolinic acid (OA) in vannamei shrimp (*Penaeus* (*Litopenaeus*) *vannamei*) after intra-sinus (10 mg/kg) and oral (10 and 50 mg/kg) administration in brackish water (salinity of 24.3 ± 2.0 ppt) at 27.2 ± 0.4°C and also investigated the net changes of OA residues in the shrimp after the thermal, acid and alkaline processing methods. The hemolymph concentrations of OA after intra-sinus dosing were best described by a two-compartment open model. The distribution and elimination half-lives were found to be 1.12 h and 34.0 h, respectively. The apparent volume of distribution at a steady state and the total body clearance were estimated to be 1,359 ml/kg and 29.9 ml/kg/h, respectively. The oral bioavailability was found to be 45.2 and 14.7% at doses of 10 and 50 mg OA/kg, respectively. The peak hemolymph concentrations after 10 and 50 mg OA/kg doses were 2.99 and 7.71 μg/ml; the time to peak hemolymph concentrations was observed at 4-h post-dosing for both groups. The elimination half-lives were found to be 34.8 and 33.4 h for the low and high dose, respectively. The residual OA was rapidly eliminated from muscle with the elimination half-life value of 23.8 and 22.5 h, respectively, for the groups treated with doses of 10 and 50 mg/kg. In shell tissues, the elimination half-life values were 24.6 and 22.5 h at dose levels of 10 and 50 mg/kg, respectively. Residual OA in muscle was reduced by around 40% by boiling, whereas it was only reduced by below 20% both by baking and frying. Residual OA in shell was reduced effectively (>60%) by boiling, acid or alkaline processes.

Keywords: *Penaeus vannamei*; Oxolinic acid; Bioavailability; Pharmacokinetics; Processing

1. Introduction

The Southeast Asia region has led the world in shrimp farming production. The black tiger shrimp (*Penaeus monodon*) was the principal species farmed there in the 1990's. In the most recent decade, shrimp farming in Southeast Asia has been undergoing a dramatic transformation. The main farmed species has rapidly been switching from *P. monodon* to vannamei shrimp (*Penaeus* (*Litopenaeus*) *vannamei*) with the introduction of specific pathogen free (SPF) *P. vannamei* broodstock (Benzie, 2009; Wyban, 2009). Nowadays in Thailand, *P. vannamei* has become the dominant cultivated species (FAO, 2013). It is an economically important export, and the greater part of the production is consumed by the USA, Japan and the EU. In 2011, the production of this species reached 511,443 metric tons (equal to approximately 1.8 billion US dollars) (FAO, 2013). However, *P. vannamei* has become susceptible to a variety of viral and bacterial diseases under intensive farming conditions with the significant increase in the production. Disease outbreak is a serious problem causing economic damage to the Thai shrimp industry. To reduce and prohibit bacterial disease problems, a wide range of antibacterial agents have been used. The misuse of antibiotics, resulting from overdosing, in shrimp farming can have an impact on the aquatic environment and has caused an increase in antibiotic resistance in bacteria (Tendencia and dela Peña, 2002). In addition, it potentially can cause human health risks due to drug residues in shrimp sold for consumption (Kleter et al., 2009). Therefore, the proper use of drugs is strongly recommended to avoid such problems. Knowledge of pharmacokinetics and residue depletion is important in order to minimize the human risk connected to drug residues and the environmental impact of the drugs. In addition, bioavailability is a key pharmacokinetic parameter which expresses the proportion of a drug administered by oral route that gains access to the systemic circulation.

Oxolinic acid (OA), a quinolone, is an antibiotic which is effective against a variety of gram-negative bacteria and which works by inhibiting the synthesis of bacterial DNA. It is one of the most commonly used antibacterial agents in Thai shrimp culture farms to treat vibriosis. There are many studies on the pharmacokinetics and bioavailability of OA in finfish species (Samuelsen, 2006), but limited information of OA dispositions in shrimp is available (Uno, 2004; Uno et al., 2006). The bioavailability of OA was reported to be only 15-40% in fish (Samuelsen, 2006) and 8-33% in shrimp (Uno, 2004; Uno et al., 2006). Most OA might be retained in the surrounding waters or sediments, owing to their low bioavailabilities in aquatic animals (Lai and Lin, 2009).

An important factor in risk assessment of antibiotic residues in shrimp is that most shrimp are usually consumed after a thermal processing such as boiling, baking and frying etc. Shrimp shells are usually not edible, but contain about 20% of chitin, poly- β (1→4)-*N*-acetyl- D-glucosamine, which has many applications in food and pharmaceutical industries (Percot et al., 2003). Currently, commercial chitin is produced from shrimp shells as by-product of the seafood industry. The extraction of chitin

from shrimp shells involves acid and alkaline processing. It is of interest to determine if drug residues are decreased by these (thermal, acid and alkaline) processes.

The objectives of the present study were to investigate the pharmacokinetics and oral bioavailability of OA in *P. vannamei* and examine the net changes of OA residues in the shrimp after the thermal, acid and alkaline processing methods.

2. Materials and Methods

2.1. Chemicals

Oxolinic acid was purchased from Sigma (St. Louis, MO, USA). Unless otherwise indicated, chemicals used were of analytical or HPLC grade.

2.2. Shrimp

Healthy *P. vannamei* (*n*=220, 26.2 ± 3.6 g) were obtained from a shrimp farm in Chanthaburi, Thailand. The shrimps were analyzed to confirm the absence of OA before the experiment. The shrimps were kept in tanks containing brackish water with 50% daily exchange at a salinity of 24.3 ± 2.0 ppt. The water temperature was 27.2 ± 0.4°C and aeration was constant. The pH of the water was 7.9 ± 0.1. The shrimps were fed *ad libitum* with commercial shrimp pellets (Blanca Feed No. 7704, Phokkaphan Aquatec Co., Chonburi, Thailand) before and after administration. The shrimps were starved for one day before administration of the drug.

2.3. Intra-sinus administration

A solution of OA for intra-sinus administration was made by dissolving OA in 0.1 M NaOH prepared in 0.9% NaCl and adjusting the pH of the solution to 10.5 with 1 M HCl. The OA concentration in the solution was 10 mg/ml. Intra-sinus administration of the drug was occurred by injection into the ventral sinus with a 50-µL Hamilton Microliter syringe 705 LT (Hamilton, Reno, NV, USA) fitted with a 27-G needle. The dose was 10 mg/kg b.w., and the injection volume was 1.0 ml/kg b.w. Five shrimps were sampled at each of 0.5, 1, 2, 4, 7, 10, 24, 48, 72, 96 and 120-h post dosing.

2.4. Oral administration

Oxolinic acid was mixed in a slurry of food. The slurry was orally administered by catheter (40 × 1.2 mm O.D.) to the shrimp. The doses were 10 and 50 mg OA/kg b.w. Five shrimps were sampled at each of 1, 2, 4, 7, 10, 24, 48, 72, 96 and 120-h post-dosing.

2.5. Hemolymph and tissue sampling

Hemolymph was sampled from the ventral sinus cavity by using a 2.5-ml syringe containing 10mg of *N*-ethylmaleimide (Sigma), used as the anticoagulant, fitted with a 21-G needle. The tail muscle and shell were collected from each shrimp. All these samples were kept frozen at -40°C.

2.6. Analytical procedures

The analytical procedures were previously described (Uno et al., 2006). Briefly, hemolymph

samples were filtered through 0.45-μm disposable syringe filter units equipped with cellulose acetate membranes (Advantec, Tokyo, Japan). The filtrate was diluted fourfold in phosphate buffered saline (PBS) and then a 20-μL portion of it was directly injected into the HPLC. Tissue samples (muscle 1 g and shell 0.5 g) were homogenized with 30 ml of 0.2% metaphosphoric acid-methanol (7:3) and centrifuged at 12,400 × *g* for 20 min. To the supernatant, 5 ml of *n*-hexane were added and vortexed. The aqueous layer was concentrated to *ca*. 10 ml at 40˚C under vacuum. The concentrated solution was poured into a Bond Elut C18 column (Varian, Harbor City, CA, USA) pre-washed with 5 ml of methanol and 10 ml of water. After the solution passed through the column, the column was washed with 10 ml of 10% methanol. The retained OA was eluted from the column with 10 ml of methanol. The eluate was evaporated to dryness. The residue was dissolved in 1 ml of 30% acetonitrile and 20-μL portion of the solution was injected into the HPLC. The HPLC system consisted of a Jasco PU-980 pump, a fluorescence detector FP-2020 (Japan Spectroscopic, Tokyo, Japan), a Rheodyne 7125 injector (Rheodyne, Cotati, CA, U.S.A.) with a 20-μL loop and a Chromatopac C-R6A integrator (Shimadzu Seisakusho, Kyoto, Japan). The operating conditions were as follows: column, Hisep column (150 × 4.6 mm I.D., Spelco, Bellefonte, PA, USA); mobile phase, 0.05 M citric acid-0.2 M disodium hydrogenphosphate-acetonitrile (70:15:15, v/v); flow rate, 1.0 ml/min; column temperature, 40˚C; and detection, FL (Ex 325 nm/Em 365 nm).

The recoveries of OA were determined from samples spiked at 1.0 μg/ml or μg/g and were 90.6 ± 1.9% (n = 5) for hemolymph, 81.3 ± 2.6% (n = 5) for muscle and 79.9 ± 1.5% (n = 5) for shell. The limits of quantitation for the spiked hemolymph, muscle and shell were 0.03 μg/ml, 0.008 μg/g and 0.02 μg/g, respectively.

2.7. Hemolymph protein binding

Hemolymph samples were taken from orally dosed shrimp at 1, 2, 4, 7, 10, 24, 48 72, 96 and 120 h. The binding of the drugs to hemolymph proteins was determined by ultrafiltration using a Microcon YM-10 (Millipore, Bedford, MA, USA) with a 10,000 nominal molecular weight cut-off limit. A 200-μl aliquot of hemolymph was used and centrifuged at 7,740 × *g* for 15 min at room temperature. The total drug concentration and free drug fraction, ultrafiltrates of hemolymph samples, were determined by HPLC as described earlier. Bound drugs were calculated as the difference between total and free components. The drug bound to the filter was determined by ultrafiltering 2 μg/ml of drug in PBS, and comparing the drug level in the filtrates and the unfiltered sample. The recovery of OA in the ultrafiltration procedure was 98.1 ± 0.8% (n = 4).

2.8. Pharmacokinetic and statistical analysis

All processes were assumed to follow first-order kinetics. Pharmacokinetic analysis was performed by the computer program WinNonlin (version 1.1, Scientific Consulting, Apex, NC, USA). Models were selected in accordance with Akaike's information criterion (Yamaoka et al., 1978). The area under the

concentration-time curve (AUC) was calculated using the trapezoid rule, including the terminal portion. The bioavailabilities (%) were estimated by calculating the AUC for oral and intra-sinus (IS) experiments and utilizing the following equation:

$$\text{Bioavailability (\%)} = (\text{AUC}_{\text{oral}} \times \text{Dose}_{\text{IS}}) / (\text{AUC}_{\text{IS}} \times \text{Dose}_{\text{oral}}) \times 100$$

The average steady-state concentration (Css) during multiple oral dosing was calculated using the following equation:

$$C_{ss} = F \times \text{Dose} / CL_b \times \tau$$

where F is the bioavailability, CL_b is the total body clearance and τ is the dosing interval.

The t-test was used for statistical comparisons; the level of significance was chosen at $P < 0.05$.

2.9. Thermal, acid and alkaline processing methods

Tissue samples (muscle and shell) were taken from orally dosed (50 mg/kg) shrimp at 24 h post-dosing. One matched tissue from each shrimp was analyzed raw for OA residue and the other was processed and analyzed. The thermal (cooking), acid and alkaline processing methods were as follows:

Boiling: Samples were wrapped in laboratory wipes (Kimwipes® S200, Kimberly-Clark, Co., USA), soaked in boiling water for 4 min, removed and cooled.

Baking: Samples were placed on a metal tray and baked in an electric oven at 200°C for 4 min, removed and allowed to cool.

Frying: Samples were fried in canola oil at 180°C for 1 min, removed and allowed to cool.

Acid treatment: Shell samples were soaked in 1M HCl at room temperature for 1 h and rinsed with distilled water. Excess water was removed.

Alkaline treatment: Shell samples were soaked in 1M NaOH solution at room temperature for 1 h and rinsed with distilled water. Excess water was removed.

3. Results

3.1. Pharmacokinetics and bioavailability

Figure 1 shows hemolymph concentrations of OA after intra-sinus administration. The hemolymph concentrations of OA could be described best by a two-compartment open model. The time course of hemolymph levels (Ct) was described by the following equation:

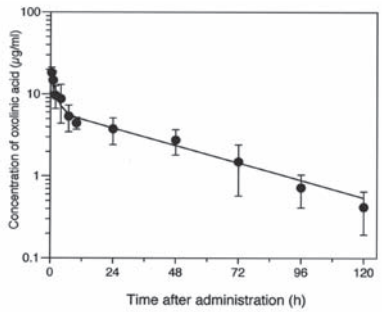

Fig. 1. Hemolymph concentrations of oxolinin acid in vannamei shrimp (*P. vannamei*) following intra-sinus administration at a dose of 10 mg/kg. Symbols indicate the mean and standard deviations for five shrimp. The line was obtained from model fitted results.

Table 1
Pharmacokinetic values for oxolinic acid in hemolymph after intra-sinus administration to vannamei shrimp (*P. vannamei*)

Parameter	Value
Water temperature (˚C)	27.2 ± 0.4
Shrimp weight (g)	26.3 ± 3.7
Dose (mg/kg)	10
C_0 (μg/ml)	22.3
A (μg/ml)	16.02
B (μg/ml)	6.28
α (h^{-1})	0.621
β (h^{-1})	0.0204
$t_{1/2\alpha}$ (h)	1.12
$t_{1/2\beta}$ (h)	34.0
AUC (μg h/ml)	334
CL_b (ml/kg/h)	29.9
V_c (ml/kg)	448
V_p (ml/kg)	911
V_{ss} (ml/kg)	1,359
$V_{d\beta}$ (ml/kg)	1,468

C_0, hemolymph drug concentration at time 0; A, B, zero-time hemolymph drug concentration intercepts of biphasic intra-sinus disposition curve. The coefficient B is based on the terminal exponential phase; α, β, values related to the slopes of distribution and terminal phases, respectively, of the biexponential drug disposition curve; $t_{1/2\alpha}$, $t_{1/2\beta}$, distribution half-life and elimination half-life, respectively, of the drug; AUC, area under the concentration-time curve from zero to infinity; CL_b, total body clearance; V_c, apparent volume of central compartment; V_p, apparent volume of peripheral compartment; V_{ss}, apparent volume of distribution at a steady state; $V_{d\beta}$, apparent volume of distribution.

$$Ct = 16.0 \times \exp(-0.621 \times t) + 6.28 \times \exp(-0.0204 \times t).$$

The distribution and elimination half-lives ($t_{1/2\alpha}$ and $t_{1/2\beta}$) were found to be 1.1 and 34.0 h, respectively. The apparent volume of distribution at a steady state (V_{ss}) and total body clearance (CL_b) were estimated to be 1,359 ml/kg and 29.9 ml/kg/h, respectively. The volume of the peripheral compartment (V_p) was obtained $V_{ss} - V_c$ (the volume of the central compartment). The V_c and V_p of OA in the shrimp were 448 and 911 ml/kg, respectively. The pharmacokinetic parameters calculated from this model are given in Table 1.

Figure 2 shows the hemolymph concentration-time profiles after oral administration at doses of 10 and 50 mg OA/kg. A non-compartment model best described data from the low and high OA dosed groups. The obtained parameters are listed in Table 2. The high dosage group resulted in 2.6-fold higher maximum concentration value when compared

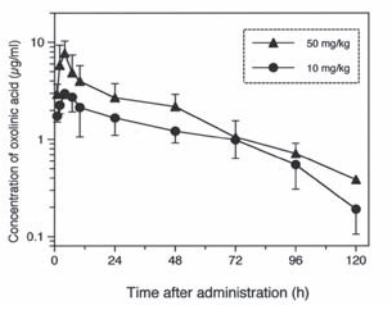

Fig. 2. Hemolymph concentrations of oxolinin acid in vannamei shrimp (*P. vannamei*) following oral administration at doses of 10 (●) and 50 (▲) mg/kg. Symbols indicate the mean and standard deviations for five shrimp.

Table 2
Pharmacokinetic values for oxolinic acid in hemolymph after oral administration to vanamei shrimp (*P. vannamei*)

Parameter	Value	
Dose (mg/kg)	10	50
Shrimp weight (g)	25.9 ± 3.4	26.4 ± 3.6
C_{max} (μg/ml)	2.99	7.71
t_{max} (h)	4.0	4.0
$t_{1/2\beta}$ (h)	34.8	33.4
AUC (μg h/ml)	151	246
MRT (h)	53.3	47.6
F (%)	45.2	14.7
Protein binding (%)	87.4 ± 5.5	84.0 ± 6.9

C_{max}, maximum concentration; t_{max}, time of maximum hemolymph concentration; $t_{1/2\beta}$, elimination half-life of the drug; AUC, area under the concentration-time curve from zero to infinity; MRT, mean residence time; *F*, bioavailability.

with that of the low dosage group, whereas the time to peak hemolymph concentration was observed at 4-h after administration for both groups. The oral bioavailability was found to be 45.2% for the low dose and 14.7% for the high dose. The hemolymph protein binding *in vivo* of OA was 87.4 ± 5.5 and 84.0 ± 6.9% at doses of 10 and 50 mg OA/kg, respectively. No significant difference was observed between the low and high OA dosed groups ($P > 0.05$).

The average steady-state concentration (C_{ss}) was calculated to be 19 μg/ml at a dosage of 10 mg OA/kg and 31 μg/ml at a dosage of 50 mg OA/kg when OA was given three consecutive daily dosings ($\tau = 8$ h).

3.2. Tissue distribution and elimination

Figure 3 shows the muscle concentration-time curves of OA after oral administration at doses of 10 and 50 mg OA/kg. The peak levels after the low and high doses were observed at 0.31 and 2.02 μg/g; the times to reach these levels were at 4-h post-dosing for the two groups. The residual OA was rapidly eliminated from muscle with the elimination half-life value of 23.8 and 22.5 h for the groups treated with doses of 10 and 50 mg OA/kg, respectively. The residual OA levels in the muscle fell below the maximum residue limit (MRL) for shrimp in Japan (0.03 μg/g) at 96 and 120-h post-dosing at dose levels of 10 and 50 mg/kg, respectively.

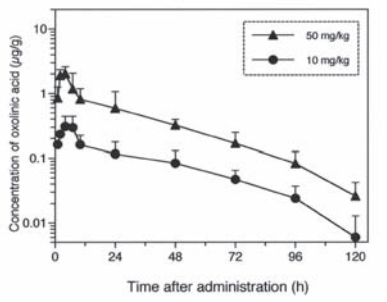

Fig. 3. Oxolinic acid concentrations in muscle tissues of vannamei shrimp (*P. vannamei*) following oral administration at doses of 10 (●) and 50 (▲) mg/kg. Symbols indicate the mean and standard deviations for five shrimp.

Figure 4 shows the shell concentration-time curves of OA after oral administration. In shell, the OA levels following the low and high doses peaked at 7 and 4-h post-dosing with peak level of 3.08 and 11.3

μg/g, respectively. The elimination half-life values in shell tissues were 24.6 and 22.5 h at dose levels of 10 and 50 mg/kg, respectively.

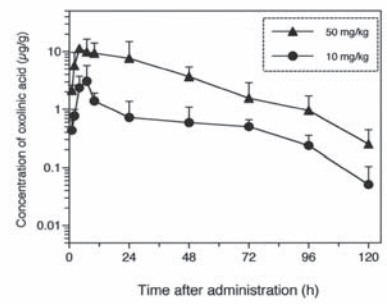

Fig. 4. Oxolinin acid concentrations in shell tissues of vannamei shrimp (*P. vannamei*) following oral administration at doses of 10 (●) and 50 (▲) mg/kg. Symbols indicate the mean and standard deviations for five shrimp.

3.3. Effect of thermal, acid and alkaline processing on the residues

Oxolinic acid residues were lower in all processing samples than in the corresponding raw samples. The residues in the processing samples were compared to those in the raw samples to evaluate the percent reduction. Figure 5 shows the substantial net reductions in OA by each of the processing methods. Residual OA in muscle was reduced by around 40% by boiling for 4 min, whereas it was only reduced by below 20% both by baking at 200°C for 4 min and frying at 180°C for 1 min. Residual OA in shell was reduced to >60% by boiling, while residual OA levels were approximately 20–30% lower in the baking and frying. Oxolinic acid residues were reduced to 60 and 69% by acid and alkaline treatment, respectively. Boiling, acid and alkaline processes were the effective treatments on the reduction of OA residues in shells.

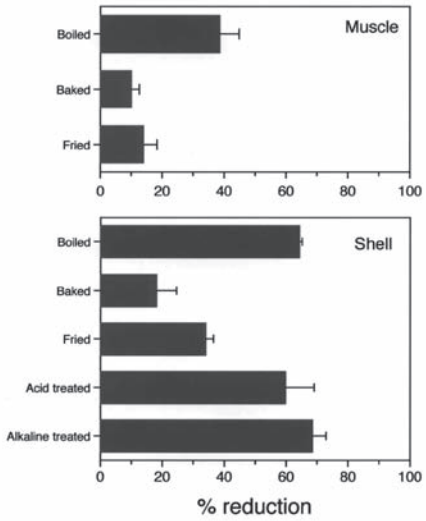

Fig. 5. Effect of thermal (boiling, baking and frying), acid (HCl) and alkaline (NaOH) processing on residual oxolinin acid in muscle and shell tissues of vannamei shrimp (*P. vannamei*). Values are the mean and standard deviations for four samples.

4. Discussion

The time course of OA hemolymph concentrations in *P. vannamei* after intra-sinus administration could be described best by a two-compartment open model with bolus injection and first-order

Table 3
Comparative pharmacokinetic values for oxolinic acid following intra-sinus administration (10 mg/kg) in penaeid shrimp

Parameter	*P. vannamei*[*1]	*P. monodon*[*2]	*P. japonicus*[*3]
α (h^{-1})	0.621	0.825	1.179
β (h^{-1})	0.020	0.039	0.021
$t_{1/2\alpha}$ (h)	1.12	0.84	0.59
$t_{1/2\beta}$ (h)	34.0	17.7	33.2
AUC/Dose	33.4	11.1	34.8
CL_b (ml/kg/h)	29.9	90.1	28.8
V_c (ml/kg)	448	723	357
V_p (ml/kg)	911	1,338	952
V_{ss} (ml/kg)	1,359	2,061	1,309
$V_{d\beta}$ (ml/kg)	1,468	2,299	1,382

[*1] This study; [*2] Uno et al. (2006), [*3] Uno (2004).

Table 4
Comparative oral bioavailability of oxolinic acid in penaeid shrimp

Parameter	*P. vannamei*[*1]	*P. monodon*[*2]	*P. japonicus*[*3]	
Dose (mg/kg)	10	50	50	50
C_{max} (μg/ml)	3.0	7.7	4.2	17.8
t_{max} (h)	4	4	4	7
F (%)	45.2	14.7	7.9	32.9
Protein binding (%)	87.4	84.0	66.2	36.7

[*1] This study; [*2] Uno et al. (2006), [*3] Uno (2004).

elimination. Previous pharmacokinetic studies of OA after intra-sinus administration have been also analyzed by a two-compartment model in kuruma shrimp (*Penaeus* (*Marsupenaeus*) *japonicus*) (Uno, 2004) and *P. monodon* (Uno et al., 2006) as well. Table 3 shows the comparative pharmacokinetic parameters of OA following intra-sinus injection (10 mg OA/kg) in penaeid shrimps. The distribution half-life ($t_{1/2\alpha}$) of OA in *P. vannamei* (1.12h) was longer than that found in *P. monodon* (0.84 h; Uno et al., 2006) and *P. japonicus* (0.59 h; Uno 2004). This indicates that OA is initially distributed more slowly to the tissues of *P. vannamei* from the hemolymph compartment than those of *P. monodon* and *P. japonicus*. The volume of the peripheral compartment (V_p) was twice as large as the volume of the central compartment (V_c) in the present study. It suggests that OA is a wide distribution, and that the major part of the drug in the body is extravascularly at distribution equilibrium. The extent of distribution (V_{ss} and $V_{d\beta}$) was similar to that in *P. japonicus*, which is smaller than that in *P. monodon*. The protein binding of a drug is related to the volume of distribution. The inverse relationship of hemolymph protein binding to the volume of distribution was demonstrated in shrimp species (Park et al., 1995; Reed et al., 2004). However, it was not observed here. In *P. vannamei* hemolymph protein binding of OA was found to be high (>80%), which was higher than that found in *P. japonicus* (36.7%; Uno 2004) and *P. monodon* (66.2%; Uno et al., 2006) (Table 4). These differences may be due to differences in the nature of hemolymph protein between penaeid species.

Elimination half-life ($t_{1/2\beta}$) and total body clearance (CL_b) are important parameters used to

characterize drug disposition. The $t_{1/2\beta}$ and CL_b of OA in *P. vannamei* hemolymph were similar to those in *P. japonicus* (Table 3). The CL_b in *P. monodon* was three times larger than that in *P. vannamei* and *P. japonicus*, and the $t_{1/2\beta}$ was half shorter than that of the two species.

In the current study, following oral administration, the maximum hemolymph concentration (C_{max}) of 50 mg/kg dose was 7.7 μg/ml, which was merely 2.5-fold higher than that of 10 mg/kg dose. Oral bioavailability (*F*) ranged from 14.7% at a dose of 50 mg OA/kg to 45.2% at a dose of 10 mg OA/kg. It demonstrates that the *F* of OA decreases with increasing doses. Previous research has also found the *F* for OA to be dose-dependent in finfish (Cravedi et al., 1987; Rogstad et al., 1993). Sangrungruang et al. (2004) reported that a high concentration of OA (50 or 100 mg/kg) dose caused serious hindgut diarrhea in *P. monodon*. Therefore, it is expected that the lowering of *F* (dose-dependent *F*) is due to diarrhea in shrimp and fish. However, the diarrhea cannot be observed apparently in the present study. Table 4 shows the comparative *F* of OA in penaeid shrimps. The *F* for OA in *P. vannamei* was twice higher than that in *P. monodon*, whereas it was half lower than that in *P. japonicus*. It is not clear whether the *F*s are influenced by their protein binding or not. Further researches are needed to elucidate the differences of *F* among the shrimp species.

In Japan, legislation stipulates maximum residue limits (MRL) for OA used in the treatment of fish and shellfish. The current MRL for OA is 0.03 μg/g in shrimp tissues, whereas it is not allocated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA). In the present study, the residual OA was rapidly eliminated from muscle and shell with a similar elimination half-life (23–25 h) at two dosages. Muscle levels fell below the MRL within 120-h post-dosing, but shell levels were above the MRL at 120-h post-dosing. Therefore, the withdrawal period should be established at longer times than 5-days.

A practical application of pharmacokinetic data is the design of clinical dosage regimens in which blood levels of a drug can be maintained within the therapeutic range by means of repeated dosages. Information on available minimal inhibitory concentration (MIC) of OA for bacterial strains associated with pathogenicity in penaeid shrimps is scant. The *Vibrio* bacteria isolated from diseased *P. monodon* in Thailand had a MIC range of 0.025–1.6 μg/ml and the inhibiting concentrations (IC) for 90% of the strains has been estimated to be 0.6 μg/ml (Ruangpan and Kitao, 1992). Hence, oral dosing of OA at a dose of 10 mg/kg (C_{ss}: 19 μg/ml) might be appropriate for use in *P. vannamei* farming in consideration of the dose-dependent bioavailability. Further investigations are needed to compare these theoretical calculations with results obtained in field situations for dosage regimens used on shrimp farms.

More information about the effect of cooking on residues is required to give a more accurate estimate of consumer exposure to these compounds, and of any breakdown products, since most food of animal origin is cooked before consumption (Rose et al., 1996). In our previous research, the residual OA was relatively heat stable in shrimp tissue under ordinary cooking procedures (Uno, 2002; Uno et

al., 2006). In the present investigation, the thermal processes could not completely degrade residual OA in shrimp muscle and shell, although boiling was more effective than baking and frying in reducing OA residues. On the contrary, OA residues in shell were considerably reduced (>60%) by the acid or alkaline treatment. In industrial processing, chitin is extracted from shrimp shells by acid treatment with hydrochloric acid to dissolve calcium carbonate followed by alkaline extraction with sodium hydroxide to solubilize proteins. The results indicate if chitin is made from shrimp shell containing detectable levels of OA, chitin products might contain negligible or near zero levels of OA.

In conclusion, the present study revealed that the pharmacokinetics of OA in *P. vannamei* was characterized by rapid absorption and wide distribution to the tissues from the hemolymph compartment, whereas it was initially distributed slowly. The residual OA was rapidly eliminated from muscle and shell with a similar elimination half-life. The oral bioavailability (F) of OA in *P. vannamei* was satisfactory (45%) at a dose of 10 mg/kg by comparison with the F at a dose of 50 mg/kg (15%). Therefore, clinical dosage regimens of OA in the shrimp should be planned taking the dose-dependent bioavailability into consideration. The thermal processing, such as cooking, could not completely degrade residual OA in shrimp tissues, whereas OA residues in shell were considerably reduced (>60%) by boiling, acid or alkaline processes.

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