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Accumulation and clearance of orally administered erythromycin in adult Nile tilapia (*Oreochromis niloticus*)

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Abstract: Nile tilapia (*Oreochromis niloticus*) were medicated with erythromycin base via the medicated ration at 50 mg and 100 mg erythromycin·kg^{-1} fish body weight^{-1}·d^{-1} for 7 days. Erythromycin residues in their muscle were determined by LC-MS/MS method through TUV Rheinland Aimex Vietnam Co., Ltd. The results predicted withdrawal time for Nile tilapia was 33 days (908°C-day) if treated by the former dose or 42 days (1150°C-day) if treated by the later one under our experimental conditions. Derivative biotransformation of erythromycin such as erythromycin C, E, F appeared in post-dosing stage, all of them were depleted to safe level if we strictly followed the withdrawal time and chemotherapeutic dosage as recommendation.

Keywords: erythromycin, tilapia, withdrawal time, LC-MS/MS, biotransformation

Introduction

Tilapias are known to have been an important component of fisheries in the Mekong River Delta. The most significant diseases in Nile tilapia (*Oreochromis niloticus*) culture are caused by *Streptococcus iniae*, *Aeromonas hydrophila*, *Trichodina*, *Flexibacter* C*olumnaris, Edwardsiella. Streptococcosis*, gram positive bacterium, has become a major problem for tilapia farmers and there is still no effective commercial vaccine available that can be used to prevent *streptococcosis* in tilapia. *Streptococcosis* can cause mass death in tilapia farms, and unlike many other tilapia diseases it will affect even large and otherwise healthy fish. The macrolide antibiotic erythromycin has long been the chemotherapeutant of choice to prevent and control *Streptococcus* spp. The objective of this study was to investigate the uptake, retention and clearance of orally administered erythromycin in tilapia *Oreochromis niloticus* given by medicated feed.

Materials and Methods

Materials

Erythromycin base in white powder and purity 96.5% was purchased from DHG Pharma (Can Tho, Vietnam).

Experimental animals and study conditions

120 adult Nile tilapias (*Oreochromis niloticus*) with an average weight 500 ± 5 g were obtained for the investigation. They were reared in farming (Ward 7, Soc Trang city, Soc Trang province, Vietnam) at pH 6.5 - 8.5, an O_2 concentration of 5 - 6 mg.liter^{-1}, and an average temperature of 28 ± 0.5°C.

Oral administration

These 120 adult Nile tilapias (*Oreochromis niloticus*) were divided into group A (60 tilapias) and group B (60 tilapias). Two different diets were prepared for the experimental trial. Group A was treated with 50 mg.kg^{-1} tilapia body weight.day^{-1} for 7 days through medicated feed (water temperature, 28°C) while group B was treated with 100 mg.kg^{-1} tilapia body weight.day^{-1} for 7 days through medicated feed (water temperature, 28°C).

Sample collection

Erythromycin base material had been estimated previously to screen and confirm whether other derivatives of erythromycin A, such as erythromycin B, C, D, E and F have been available or not.

Sampling times for the fish in group A and group B were 1, 3, 6, 9 and 23 days post-dosing. At each sampling time, five fishes of each group were sacrificed to confirm erythromycin A residue. Meanwhile, bio-transformation of erythromycin in tilapia was monitored by screening and confirming derivative forms of erythromycin at the beginning and the end of sampling stage.

Muscle samples in natural proportion were

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collected, and placed into polyethylene bags, coded and transferred to the laboratory on dry ice, stored at -40°C before analysis.

Analytical procedures

The methodology used for the determination of erythromycin A as well as derivatives of erythromycin in erythromycin base material, in fish muscle was based on LC-MS/MS method via TUV Rheinland Aimex Vietnam Co., Ltd. certified by DIN EN ISO/IEC 17025:2005 from DGA (German accrediation).

Results and Discussions

Clearance of erythromycin A

In order to consider the influence of water temperature on fish metabolism and, consequently, on the drug pharmacokinetics, the time parameter was also expressed as °C-day. Degree-days were calculated by multiplying the mean daily water temperature by the total number of days at which the temperature was measured to that point.

Results of erythromycin A depletion at different times in tilapia samples treated with 50 and 100 mg kg^{-1} fish body weight day^{-1} for 7 days were shown in table 1 and table 2, respectively.

Figure 3 and 4 showed the data reported in Table 1 and 2, displayed here on a semilogarithmic graph, where singular animal data were plotted. On the y axis, erythromycin concentration (µg/kg) was plotted, while on the x axis, time post treatment (degree-days) was shown. The MRL value for erythromycin was set at 30 $\mu g.kg^{-1}$, as reported by CFIA (Canadian Food Inspection Agency), date 17/11/2009. The regression line and the upper, one-sided tolerance limit (95%) regression line with a confidence of 95% were also traced. This graph had been obtained using the statistical program recommended by the European Agency for the Evaluation of Medicinal Products (EMEA). Using this statistical method, a withdrawal time of 908°C-days was interpolated for tilapia treatment, with 7 days of 50 $mg.kg^{-1}$ fish body weight. day^{-1} erythromycin. Meanwhile a withdrawal time of 1150°C-days was interpolated for tilapia treatment, with 7 days of 100 $mg.kg^{-1}$ fish body $weight.day^{-1}$ erythromycin.

Bio-transformation of parent drug

Our research was conducted in conditions that were quite close to actual aquaculture. In erythromycin base powder, erythromycin F presented at 5 µg/kg in concentration.

Salmon Oncorhynchus mykiss, after its erythromycin administration at 100 $mg.kg^{-1}$ trout body $weight.day^{-1}$ for 21 days through medicated feed (water temperature, 11.5°C) gave a withdrawal time of 255°C-days (Annarita et al., 2007). Salmon Oncorhynchus tshawytscha through intraperitoneal injection (William, 2006) as well as orally administered erythromycin (Fairgrieve, 2005), the mechanism of its retention and depletion was also investigated. The high metabolic rate of furazolidone, AOZ in Nile tilapia was 22 days at least (Weihai, 2006). Meanwhile, a research of accumulation and clearance of florfenicol in tilapia didn't rule out the withdrawal times (Bowser, 2009).

When tilapias were medicated with erythromycin base at low dose (group A), none of derivatives of erythromycin was detected in tilapia muscle at day 1 of post-treatment. At day 23 of post-treatment, erythromycin E (0.30 µg/kg) and erythromycin F (1.37 µg/kg) was not significant to our concern.

In case tilapias were fed with erythromycin at higher dose (group B), two derivatives erythromycin C (131.5 µg/kg) and erythromycin E (258.3 µg/kg) appeared right after ceasing drug treatment. This phenomenon could be explained by intestinal and hepatic enzymes. Maltase, leucine aminopeptidase, dipeptidyl aminopeptidase IV, lipase, non-specific esterases, and alkaline phosphatase were their intestinal enzymes participated in erythromycin metabolism.

Bundit et al. (2000) showed that maltase, leucine aminopeptidase, dipeptidyl aminopeptidase IV, lipase, non-specific esterases, and alkaline phosphatase were present at specific sites along the first four intestinal segments. Strong reaction for maltase was present in the third intestinal segment, while aminopeptidases and alkaline phosphatase were detected in the first three parts. The most intense activity for lipase was present in the first two parts, while non-specific esterases were observed in the first four portions. Activities of all these enzymes were demonstrated in the brush border. Non-specific esterases were also present in the cytoplasm of the enterocytes. In addition to its brush border localization in the cranial segments, dipeptidylaminopeptidase IV was also observed in the basal lamina of all segments, including the terminal segment. The first four regions played the most important role in both digestion and absorption of erythromycin.

Parallel with intestinal enzymes, hepatic biotransformation enzymes in tilapia such as CYP1A protein, 7-ethoxyresorufin O-deethylase (EROD), glutathione S-transferase (GST), UDP-glucuronasyl transferase (UDP-GT) and lipogenic enzyme were also dominated and highly correlated with erythromycin demethylase (Bernard,1996). They eliminated erythromycin derivatives to minor level at

Table 1. Erythromycin A clearance at different times in tilapia fillet samples treated with 50 mg.kg^{-1} fish body weight. day^{-1} for 7 days

Time		Erythromycin residue in tilapia fillet (µg/kg) [α]
Day	°C – Day	
1	28	22,216.0 ± 22,023.0
3	84	13,590.0 ± 14,415.9
6	168	940.8 ± 460.3
9	252	131.4 ± 31.9
23	644	34.7 ± 9.6

[α]Values shown are concentration means ± standard deviations from 5 tilapia fillet samples

Table 2. Erythromycin A clearance at different times in tilapia fillet samples treated with 100 mg kg^{-1} prawn body weight day^{-1} for 7 days

Time		Erythromycin residue in tilapia fillet (µg/kg) [α]
Day	°C - Day	
1	28	46,960.0 ± 9,054.7
3	84	14,328.0 ± 18,336.1
6	168	6,382.0 ± 5,582.5
9	252	379.7 ± 99.3
23	644	42.9 ± 17.4

[α]Values shown are concentration means ± standard deviations from 5 tilapia fillet samples

Table 3. Derivative forms of erythromycin at different times in tilapia fillets treated with 50 mg.kg^{-1} fish body weight.day^{-1} for 7 days

Name of Sample	Identification	Test parameter	MDL (µg/kg)	Result (µg/kg)
Erythromycin Base	EBS/0901-RC-002	Erythromycin B	1.0	N.D
		Erythromycin C	1.0	N.D
		Erythromycin D	1.0	N.D
		Erythromycin E	1.0	N.D
		Erythromycin F	1.0	5.00
Tilapia Fillet	TL - S1	Erythromycin B	10.0	N.D
		Erythromycin C	10.0	N.D
		Erythromycin D	10.0	N.D
		Erythromycin E	10.0	N.D
		Erythromycin F	10.0	N.D
Tilapia Fillet	TL - S5	Erythromycin B	10.0	N.D
		Erythromycin C	10.0	N.D
		Erythromycin D	10.0	N.D
		Erythromycin E	10.0	0.30
		Erythromycin F	10.0	1.37

Table 4. Derivative forms of erythromycin at different times in tilapia fillets treated with 100 mg.kg^{-1} fish body weight.day^{-1} for 7 days

Name of Sample	Identification	Test parameter	MDL (µg/kg)	Result (µg/kg)
Erythromycin Base	EBS/0901-RC-002	Erythromycin B	1.0	N.D
		Erythromycin C	1.0	N.D
		Erythromycin D	1.0	N.D
		Erythromycin E	1.0	N.D
		Erythromycin F	1.0	5.00
Tilapia Fillet	TL - SC1	Erythromycin B	10.0	N.D
		Erythromycin C	10.0	131.49
		Erythromycin D	10.0	N.D
		Erythromycin E	10.0	258.28
		Erythromycin F	10.0	N.D
Tilapia Fillet	TL - SC5	Erythromycin B	10.0	N.D
		Erythromycin C	10.0	N.D
		Erythromycin D	10.0	N.D
		Erythromycin E	10.0	6.94
		Erythromycin F	10.0	5.90

** N. D: Not detected*

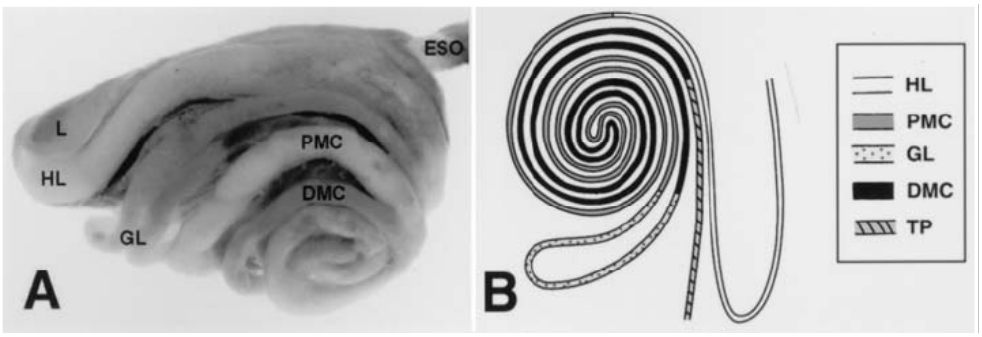

Figure 1. Photomicrograph (right view) (A) and schematic drawing (ventral view) (B) of five intestinal segments of tilapia. HL, hepatic loop; PMC, proximal major coil; GL, gastric loop; DMC, distal major coil; TP, terminal portion of the intestine

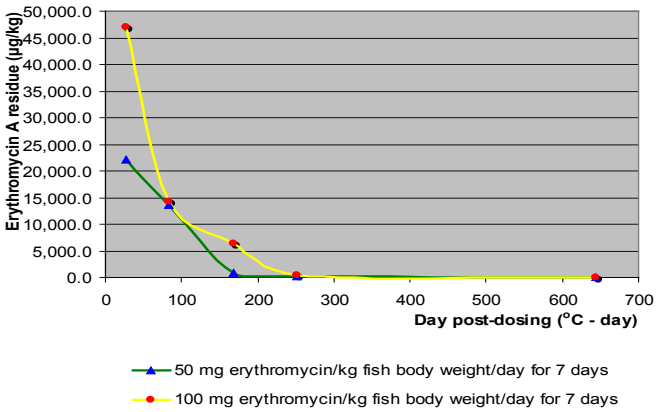

Figure 2. Erythromycin A clearance in tilapia fillet samples treated with 50 & 100 mg.kg^{-1} fish body weight. day^{-1} for 7 days

Determination of withdrawal time

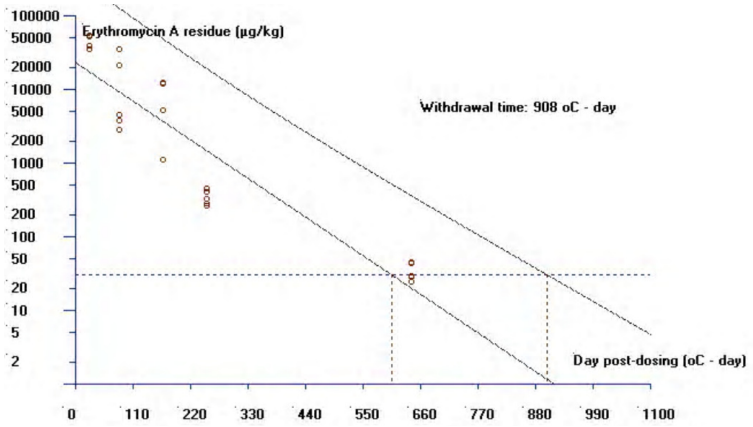

Figure 3. Linear regression line and upper one-sided tolerance limit (95%) linear regression line, with a confidence of 95%, of erythromycin concentrations in muscle tilapia treated with erythromycin for 7 days (50 mg.kg^{-1} fish body weight.day^{-1}) versus time. Degree-days are calculated by multiplying the mean daily water temperatures by the total number of days measured.

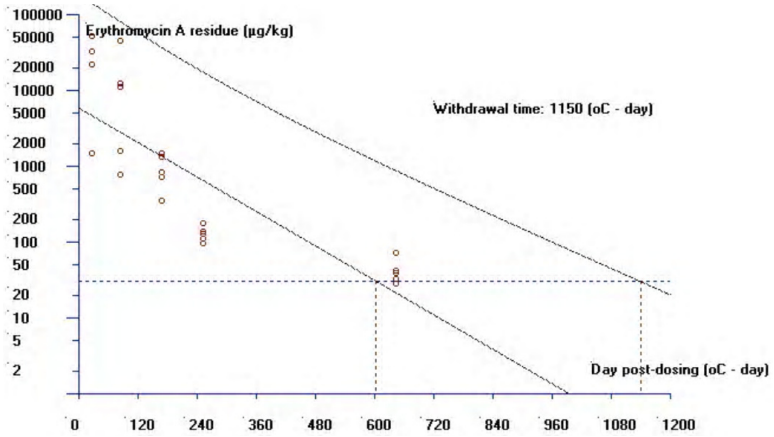

Figure 4. Linear regression line and upper one-sided tolerance limit (95%) linear regression line, with a confidence of 95%, of erythromycin concentrations in muscle tilapia treated with erythromycin for 7 days (100 mg.kg^{-1} fish body weight.day^{-1}) versus time. Degree-days are calculated by multiplying the mean daily water temperatures by the total number of days measured.

date 23 post-dosing.

Conclusion

In conclusion, our study provides preliminary data for a prudent use of the antimicrobial drug erythromycin in Nile tilapia, in order to guarantee safety in foods for the consumers and to improve fish farming management. The withdrawal time of erythromycin in Nile tilapia was recommended 33 days or 42 days at least depend on dosage of chemotherapy.

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References

Annarita, E., Laura, F., Dario, L., Luigi, M., Ettore, C. and Emilio, G. 2007. Orally administered erythromycin in rainbow trout (Oncorhynchus mykiss): Residues in edible tissues and withdrawal time. Antimicrobial Agents and Chemotherapy 51: 1043–1047.

Bernard, K.-M.G., Marian, A. and Anders, G. 1996. Species characteristics of hepatic biotransformation enzymes in two tropical freshwater teleosts, tilapia (Oreochromis niloticus) and mudfish (Clarias anguillaris). Comparative Biochemistry and Physiology Part C: Pharmacology, Toxicology and Endocrinology 114: 201-211.

Bundit, T., Bonnie, J. S., Thomas, C. and Stephen, A. S. 2000. Distribution of intestinal enzyme activities along the intestinal tract of cultured Nile tilapia, Oreochromis niloticus L. Aquaculture 182: 317–327.

Fairgrieve, W.T., Masada, C.L., McAuley, W.C., Peterson, M.E., Myers, S. and Strom, M.S. 2005. Accumulation and clearance of orally administered erythromycin and its derivative, azithromycin, in juvenile fall Chinook salmon Oncorhynchus tshawytscha. Diseases of Aquatic Organisms 64: 99-106.

Nagla. F. Galal, Safinaz G.M. Ismail, R.H.Khalil, M.K. Soliman, 2005. Studies on Edwardsiella infection in Oreochromis niloticus. Egyptian Journal of Aquatic Research 31: 460 – 471.

P. R. Bowser, R. E. Kosoff, C-Y. Chen, G. A. Wooster and R. G. Getchell, 2009. Florfenicol residues in Nile tilapia after 10-d oral dosing in feed: effect of fish size. Journal of Aquatic Animal Health 21: 14-17.

Weihai, X., Xiaobin, Z., Xinting, W., Liping, D., Gan, Z. 2006. Residues of enrofloxacin, furazolidone and their metabolites in Nile tilapia Oreochromis niloticus. Aquaculture 254: 1-8.

William, T. F., Cyndy, L. M., Mark, E. P., Carlin, M., Gail, C. M. and Mark, S. S. 2006. Concentrations of erythromycin and azithromycin in mature Chinook salmon Oncorhynchus tshawytscha after intraperitoneal injection, and in their progeny. Diseases of Aquatic Organisms 68: 227 – 234.