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ORIGINAL ARTICLE

Pharmacokinetics of Florfenicol Administrated to Rainbow trout (*Oncorhynchus mykiss*) by oral gavages and medicated feed routes

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ABSTRACT

The most cultivated freshwater species is rainbow trout in aquaculture worldwide.This study was aimed at pharmacokinetics of florfenicol administrated to rainbow trout (Oncorhyncus mykiss) by oral gavages (through a tube leading down the throat to the stomach) and medicated feed. 132 healthy rainbow trout weighing 140±10g were randomly selected and after 2 weeks acclimation period,florfenicol were administrated as oral gavages and medicated feed at single dose 10 mg/kg^{-1} body weight (B.W) to individual fish. Plasma samples were collected at 0, 0.5, 1, 3, 6, 9, 12, 24, 36, 48 and 72 h after feeding and analyzed by high performance liquid chromatography (HPLC)method. The data obtained from plasma concentrations of florfenicol after oral gavages and feed medicated routes were analyzed by SPSS version 16, Mann-Whitney U, ANOVA tests and (P < 0.05) was considered significant. The maximum concentration (C_{max}) was gained at 12 h for gavages route (4.68µg/ml^{-1}), but at 9 h for medicated feed (6.1µg/ml^{-1}). Themaximum level concentration of florfenicol in medicated feed route was higher than oral gavages route (P < 0.05). It seems that feed can increase absorbance of florfenicol. Also, interestingly the C_{max} in medicated feed route rapidly reached and the decreasing process of drug showed less elimination in versus of time.

Keywords: Rainbow trout, Florfenicol, Pharmacokinetics, Gavages route, medicated feed route

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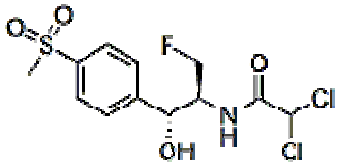
INTRODUCTION

The most cultivated freshwater species is rainbow trout in aquaculture worldwide. Fish production is annually increasing in Europe, Asia and American continents [1]. Germany, France, Italy, Iran and United States are countries which large production of fish has been registered [2]. In aquaculture, bacterial diseases are common with high density, so for prophylaxis or treatment of disease, the use of antibacterial agents is inevitable (3).

In the past, one of the main antibacterial agents in aquaculture was chloramphenicol which due to bone-marrow depression and irreversible aplastic anemia in human, its use was limited until florfenicol, fluorinated analogue of thiamphenicol (Fig. 1), synthesized and approved for veterinary use [4].

Figure 1.Chemical structure of florfenicol (fluorinated analogue of thiamphenicol)



Improvement of florfenicol had been done for use in fresh water-reared salmonids at 2007 by the U.S. Food and Drug Administration Centre for Veterinary Medicine. Florfenicol has great potential for treatment of bacterial infections of fish [5]. It is more active than thiamphenicol, chloramphenicol, and dangerous bacteria such as *Aeromonassalmonicida, Vibrio Salmonicida,* and *Edwardsiellaictaluri* are susceptible to florfenicol. Oral administration of florfenicol for treatment of bacterial infections of captive fish has been done under the trade names of Aquaflor® and Aquafen® in Canada and Europe, respectively [6]. Oral administration of florfenicol could be either as oral solution or medicated feed. Several studies have been done about pharmacokinetics of florfenicol in trout as oral and intramuscular administrations [7, 8], but this study was aimed at pharmacokinetics of florfenicol following gavage and medicated feed administrations in rainbow trout.

MATERIALS AND METHODS

Chemicals

Florfenicol standard was purchased from Sigma chemicals Co., USA and chloramphenicol standard was purchased from Merck Co, Germany. Stock standard solutions of florfenicol and chloramphenicol were prepared as 1mg/ml^{-1} and 0.5mg/ml^{-1} by dissolving each drug in methanol,respectively and stored at -20˚C.

Fish

132 healthy rainbow trout (*Oncorhynchusmykiss*) weighing 140±10 g obtained from a trout breeding centre (2000 center, Tonekabon, Iran) were reared in fresh water. The fish were brought to cement tanks of Coldwater Fishes Research Centre which was disinfected 1 day prior to transmission. Water constant flow of 720 L/h^{-1} with oxygen content of (92±2) %, PH (7.2±0.1) and temperature of 14-15˚C in cement tanks were established. The fish were fed with pellet in amount of 2% of the body weight TID for 2 weeks. After acclimation period (2 weeks), the fish were staved for 1 day before administration of drug.

Drug and administration route

Florfenicol powder was donated by Behvazan Company (Tehran, Iran). Oral suspension of florfenicol (2.5mg ml^{-1}) was prepared by dissolving florfenicol powder in propylene glycol. Medicated feed was prepared by blending the drug in feed (10mg/g^{-1}feed).A single dose of 10 mgkg-1body weight (B.W) florfenicol was given to individual fish.

Sampling

The samples were taken at 0, 0.5, 1, 3, 6, 9, 12, 24, 36, 48 and 72h after drug administration (oral gavages and medicated feed route).Blood samples were taken from caudal vein using a heparinized 2.5 ml syringe and plasma was isolated by centrifugation at 3000 rpm for 10 min. all samples were instantly frozen and stored at 20˚C until analysis.

Sample preparation

0.5ml plasma sample was added to 30µl chloramphenicol (2µg/ml^{-1}) for use as the internal standard. Each sample was whirl mixed for 2 min and then 3.5ml ethyl acetate was added and centrifuged at 3500 rpm for 1min to precipitate proteins. 2.5 ml supernatant was removed and evaporated to dryness under a gentle steam of nitrogen at 40˚C. The residue was dissolved in 0.5ml of mobile phase solution (water - acetonitrile, 75:25, v/v) and after centrifugation, filtered through a syringe filter (0.45µm), and 100µl were injected on the HPLC column.

Chromatographic condition

The analyses were performed by HPLC system (waters 2695, U.K), consisted of a reaction pump, Intelligent pump and waters 486 detector at 234 nm. The separation was performed at 40˚C on a 200 mm × 4.6 mm I.D. ODS-A column packed with 5 µm, 120-A C18 stationary phase. The mobile phase was water-acetonitrile (75, 25 V/V) which filtered through a 0.45 µm filter and degassed by sonication (5 min).The flow rate was 2.5ml/min^{-1}.

Analyze quantification

Working standard solutions (0.1, 0.25, 0.5, 1, 2.5, 5, 10, 20 and 25) encompassing the expected concentrations of florfenicol were injected on the HPLC system to generate a calibration curve with coefficient of determination (r^2) exceeding 0.997. The recovery rate for florfenicol was 92.3-98.1% .The limit of quantification was 0.03µg/gr^{-1}.

Statistical analysis

The data obtained from plasma concentrations of florfenicol after gavage and medicated feedroutes were analyzed by SPSS version 16, Mann-Whitney U, ANOVA tests and ($P< 0.05$) was considered significant.

RESULTS AND DISCUSSION

In the present study results show that the mean concentrations of florfenicol in plasma versus time were shown in table 1.

Table 1: Concentrations of florfenicol in plasma after oral gavages and medicated feed routes

Oral administration	Time(h)									
	0.5	1	3	6	9	12	24	36	48	72
Gavage route(μg/ml^{-1})	1.66	2.21	2.96	2.1	3.1	4.68	1.34	1.91	0.78	0.49
Medicated feed route(μg/ml^{-1})	1.6	3.1	4.2	5.6	6.1	4.9	3.1	1.58	0.93	0.79

Plasma concentration of florfenicol after 0.5 h reached 1.66μg/ml^{-1} and maximum plasma concentration (4.68μg/ml^{-1}) was gained at 12 h for oral gavages route but plasma concentration for medicated feed route after 0.5 h reached 1.60μg/ml^{-1} and maximum plasma concentration was 6.1μg/ml^{-1}at 9h. The drug level steadily decreased and reached 0.49μg/ml^{-1}, 0.79μg/ml^{-1} for oral gavages and medicated feed administrations after 72 h, respectively. Pharmacokinetics parameters for florfenicol are shown in table 2.

Table 2: Pharmacokinetics parameters for florfenicol after oral gavages and medicated feed routes

	Dose	C_{max}	T_{max}
Oral Administration	$mgkg^{-1}$	μgml^{-1}	h
Gavage route	10	4.68	12
Feed medicated route	10	6.1	9

Plasma concentrations of florfenicol in medicated feed route were significantly higher than plasma concentration of florfenicol in gavages route (*P < 0.05*).

Several studies have performed on pharmacokinetic of florfenicol in different fish species. Oral administration is common route of drug administration in fish (8 & 9). This study was done to evaluate the pharmacokinetic of florfenicol following gavage and medicated feed administration in rainbow trout. Maximum level concentration in gavage rout reached after 12h (4.68μg/ml^{-1}) but maximum level concentration in feed medicated rout reached after 9h (6.1μg/ml^{-1}) with dose of 10mg/kg^{-1}. Feng et al. (6) have reported maximum level concentration in oral rout in tilapia 4.46μg? ml^{-1}at 22 ˚C which is in agreement with our study in oral gavages route.

The maximum level concentration of florfenicol in medicated feedroute was higher than gavage route .It seems that feed can increase absorbance of florfenicol.

Also, interestingly the maximum level concentration in medicated feed route rapidly reached. Meinertz et al. (10) have reported florfenicol concentration in skin-on fillets in the recirculating aquaculture system (RAS) 11.58μg/ml^{-1} at 13˚C. T_{max} of 12h in plasma in oral gavages route was in agreement with the results of Feng et al. (6) in tilapia and Martinsen et al. [11] in Atlantic salmon after oral dosing at 10mg/kg^{-1}florfenicol. In this study, the decreasing process of drug in medicated feed route showed less elimination in versus of time. Martinsen et al. [11] have reported rapid absorption andless elimination in medicated feed. Feed medicated route is a safe method with high efficiency which is confirmed by Straus et al. (12) and is in agreement with our study. Cao et al. [13] have reported C_{max}10.8μg/ml^{-1} after 8 h in top mouth culter (*Culteralburnus*) which was higher than our results. In conclusion, the present study showed that florfenicol as medicated feed route was better than gavage route both higher and rapid concentration in rainbow trout.

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