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Pharmacokinetics of flumequine and *in vitro* activity against bacterial pathogens of gilthead sea bream *Sparus aurata*

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ABSTRACT: The present study investigated the kinetic profile of flumequine (FLU) in gilthead sea bream *Sparus aurata* (170 g) held at 19°C and evaluated its *in vitro* efficacy against important bacterial diseases in Mediterranean mariculture. Following a single intravascular injection (10 mg kg^{-1} fish), the distribution half-life ($t_{1/2\alpha}$) and the half-life of the terminal phase of elimination ($t_{1/2\gamma}$) of the drug were 0.2 and 30 h respectively. Tissue penetration of FLU was low, since both the apparent distribution volume of the drug at steady-state ($V_{d(ss)}$) and the apparent volume of the central compartment (V_c) were small (0.57 and 0.15 l kg^{-1}). The mean residence time (MRT) was short (11 h) and the total clearance (CL_T) of the drug was slow (0.05 l kg^{-1} h^{-1}). Following oral administration (20 mg kg^1), the bioavailability (F %) of FLU was 29% and the maximum plasma concentration was 1.7 µg ml^{-1}. The minimum inhibitory concentration (MIC) of the drug in distilled water supplemented with 2% NaCl against *Vibrio anguillarum* Serotype 1b, *Photobacterium damsela* ssp*. piscicida*, *V. alginolyticus*, *V. damsela* and *V. fluvialis* was 0.15, 0.3, 1.2, 0.019 and 0.15 µg ml^{-1} respectively. The addition however of 10 mM Ca^{2+} and 55 mM Mg^{2+} to the medium resulted in an 8- to >120-fold reduction in FLU activity. The results indicate that FLU has an adequate kinetic profile in gilthead sea bream and that marine cations induce a significant impact on the activity of FLU, rendering its use against bacterial pathogens questionable.

KEY WORDS: Flumequine · Gilthead sea bream · Pharmacokinetics · Bioavailability · Minimum inhibitory concentration · Mediterranean mariculture

INTRODUCTION

Bacterial pathogens remain a considerable threat in fish farming, causing economic losses. Therefore, antibacterial drugs are of importance, particularly in diseases for which vaccines have not yet been developed. The continuous increase of fish farming worldwide has inevitably been accompanied by an extensive and occasionally improper use of antibacterial agents to confront these pathogens. This may have undesired consequences such as the emergence of bacterial pathogens resistant to the relative paucity of registered antibacterial agents. Consequently, the need for new agents with attractive chemotherapeutic properties in farmed fishes is of importance. Evaluation of the efficacy of an antibacterial agent is based on its activity against important bacterial pathogens, which is mainly calculated as the minimum inhibitory concentration (MIC) coupled with a measurement of its plasma levels in the target species. A fast kinetic profile of the candidate drug is desirable because treated fishes must be free from residues before marketing.

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The inclusion of such data would provide a complete picture of the clinical outcome, and possibly enhance the efficacy of the treatment and secure human health and the environment.

Flumequine (FLU) is a fluoroquinolone derivative which inhibits DNA-gyrase (Drlica & Coughlin 1989). It is a broad-spectrum synthetic antibacterial agent mainly against Gram-negative bacteria. Previous studies have revealed that FLU induces satisfactory kinetic properties in some farmed fish species (Rogstad et al. 1993, Martinsen & Horsberg 1995) and low MICs against important bacterial fish pathogens (Ledo et al. 1987, Martinsen et al. 1992), indicating that FLU is a promising therapeutic candidate for fish farming.

Ample information exists on the pharmacokinetics of FLU in coldwater fishes including the rainbow trout *Oncorhynchus mykiss* (Sohlberg et al. 1990, 1994), the European eel *Anquilla anguilla* (Boon et al. 1991, van der Heijden et al. 1994), the common carp *Cyprinus carpio* (van der Heijden et al. 1994), the Atlantic salmon *Salmo salar* (O'Grady et al. 1988, Rogstad et al. 1993, Elema et al. 1994, 1995, Martinsen & Horsberg 1995), the Atlantic halibut *Hippoglossus hippoglossus* (Samuelsen & Lunestad 1996, Samuelsen & Ervik 1997, Hansen & Horsberg 1999), the turbot *Scophthalmus maximus* (Hansen & Horsberg 1999) and the channel catfish *Ictalurus punctatus* (Plakas et al. 2000).

A first attempt to determine the pharmacokinetics of FLU in warmwater-farmed fishes was performed on the sea bass *Dicentrarchus labrax* without including bioavailability data or MICs (Rigos et al. 2002). Additionally, the kinetic profile of FLU has previously revealed significant interspecific differences (van der Heijden et al. 1994), an indication that the extrapolation of treatment regimes and withdrawal times for target fishes using data derived from other species is improper and may result in false therapies and environmental pollution.

The aim of the present study was to provide additional information on the pharmacokinetic properties of FLU in the gilthead sea bream *Sparus aurata*, the main euryhaline farmed species, and to determine MICs against important bacterial pathogens of Mediterranean mariculture.

MATERIALS AND METHODS

Chemicals. FLU was obtained from Sigma Chemical. The chemicals used for processing the plasma samples (HPLC grade) were obtained from Labscan. Intravenous and oral doses of flumequine were dissolved in 0.1 M NaOH (pH was adjusted to 10.5 with HCl). During the experiments, fish were anaesthetised with quinaldine (Sigma Chemical).

Experimental design. Healthy gilthead sea bream *Sparus aurata* averaging 170 g were acclimatised for 2 wk prior to initiation of the study (facilities of the Laboratory of Fish Nutrition and Pathology, National Centre for Marine Research, Athens). The fish were maintained in cylindroconical fibreglass tanks (800 l) receiving 36‰ sea water at a temperature of 19 ± 0.5°C, and were starved for 2 d before the start of the experiment; they remained unfed during the study.

Intravascular injection. Prior to injection, the fish were anaesthetised (2 ml l^{-1}) and weighed. They received 200 µl of medicated solution at a dose of 10 mg kg^{-1} fish. To ensure the correct position of the needle (Microlance 23G $1_{1/4}$ 0.6 × 30, Becton Dickinson SA) in the caudal vein, blood was aspirated into the syringe prior to and following injection. Fish that bled heavily were removed and replaced. Approximately 1 ml of blood was drawn from the caudal vein (some distance from the injection site) of several fish (4 to 5 individuals sampled once), at each of the time points following dosing (0.5, 1, 4, 8, 16, 24, 48, 70 and 100 h). Plasma was prepared from blood samples by centrifugation at 3500 × *g* for 10 min at 4°C. Plasma samples were stored at –20°C until analysis.

Oral administration. Prior to oral administration of the drug, the fish were anaesthetised (2 ml l^{-1}) and weighed. Previous experience with single drug administrations in their feed has shown considerable variation in the drug plasma concentration of treated fish; therefore FLU (20 mg kg^{-1} fish) was administered via a stomach tube (diameter 2 mm) in sterile aqueous suspensions (0.4 ml) containing the red food stain erythrosine to detect eventual regurgitation of the drug. Fish which regurgitated the drug were excluded from the study. Approximately 1 ml of blood was taken from the caudal vein of several fish (4 to 5 individuals) at each of the time points following dosing (2, 4, 8, 16, 24, 48, 70 and 100 h). Plasma preparation and storage conditions were identical to the protocol used for intravascular administration.

FLU analysis. The determination of FLU in plasma samples followed the methodology of a previous study (Rigos et al. 2002). Briefly, extraction of the drug in plasma samples (200 µl) was conducted with 2 ml ethyl acetate. Samples were vortexed and centrifuged at 5000 × *g* before being evaporated with N_2 at 50°C. Resuspension of the drug was performed with 1 ml of mobile phase (50% acetonitrile:methanol [3+2] v:v, 50% trifluoroacetic acid 0.1%, pH 2.15). The samples were filtered (0.22 µm) before injecting 20 µl thereof into the HPLC system (ALLIANCE 2690 MX, Waters™) used for the detection of FLU, which consisted of a pump (Waters™ 510), an autosampler (Waters™ 717 plus), a column (ZORBAX$^{\alpha}$ SB–C18, 5 µm [250 × 4.6 mm]) and a scanning fluorescence detector (Waters™ 474). The excitation wavelength

was set at 327 nm and emission at 369 nm. The flow rate was adjusted at 0.8 ml min^{-1} and the retention time was 7.5 min. A standard curve for flumequine (10, 50, 150 and 750 ng ml^{-1}) in triplicate drug-free plasma samples was conducted ($r^2 = 0.997$). Oxolinic acid was used as internal standard. The percentage of recovery (75%) was calculated by comparing FLU concentration in spiked plasma samples to a standard solution in 0.1 M NaOH. The detection limit was 10 ng ml^{-1}.

Pharmacokinetic analysis. The plasma data of the intravascular injection was analysed for best fit to a 2- or 3-compartment open pharmacokinetic model by using non-linear regression analysis (NLREG 2001, PH Sherrod), following a semi-logarithmic plot of the data (curve EXPERT 1997, D Hyams) and least-square fitting (Ritschel 1986). The plasma data were better fitted to a 3-compartment open pharmacokinetic model (Fig. 1). The plasma concentration versus time curve was calculated from the 3-exponential equation:

$$C = Ae^{-\alpha t} + Be^{-\beta t} + \Gamma e^{-\gamma t}$$

where C is the plasma concentration, t is the time, α, β and γ are the slopes of mono-exponential declining curves, and A, B and Γ are the zero time plasma concentrations. The diffusion processes were all assumed to follow first-order kinetics. Calculations of apparent volume of distribution at steady state ($V_{d(ss)}$) and total body clearance (CL_T) were performed in a model-independent way (Ritschel 1986). The area under the concentration-time curve (AUC) was calculated using the trapezoidal rule (Ritschel 1986) and was extrapolated to infinity. The half-lives were calculated from the equation $t_{1/2} = \ln 2/\alpha$, β or γ. The slope β of the plasma data following oral administration used to extrapolate AUC to infinity was calculated from the last 4 time points of the elimination curve ($r^2 = 0.98$). Bioavailability (F%) was estimated according to the equation:

$$F = \frac{(\text{AUC oral administration}) \times (\text{dose intravascular injection}) \times 100}{(\text{AUC intravascular injection}) \times (\text{dose oral administration})}$$

MIC determinations. MICs were determined by the broth microdilution method at 22°C. An antibiotic solution was made by dissolving FLU in distilled water along with 1 M NaOH. Dilutions were made in Mueller Hinton broth (MHB) dissolved in either distilled water supplemented with 2% NaCl or with 10 mM Ca^{2+} and 55 mM Mg^{2+} (which approximates Mediterranean salinity), since previous studies have stressed the impact of these cations on the activity of drugs in the marine environment (Barnes et al. 1995, Pursell et al. 1995). FLU concentrations in the test wells ranged from 0.019 to 38.25 µg ml^{-1}.

The bacteria which inflict the most significant losses in euryhaline mariculture, comprising the primary pathogens *Vibrio anguillarum* Serotype 1b and *Photobacterium damsela* ssp. *piscicida*, and the opportunistic bacteria *V. alginolyticus*, *V. damsela* and *V. fluvialis*, were acquired from the Fish Pathology Laboratory of the National Centre for Marine Research, Greece. All bacterial pathogens were derived from diseased farmed gilthead sea bream and sea bass from 2000 to 2001. Since, in Greek mariculture, disease cases related to these bacteria deal mainly with 1 isolate of each species (90% frequency), only 1 representative strain (see Table 3) of each bacterial pathogen was tested. The *Vibrio* species and *P. damsela* ssp. *piscicida*

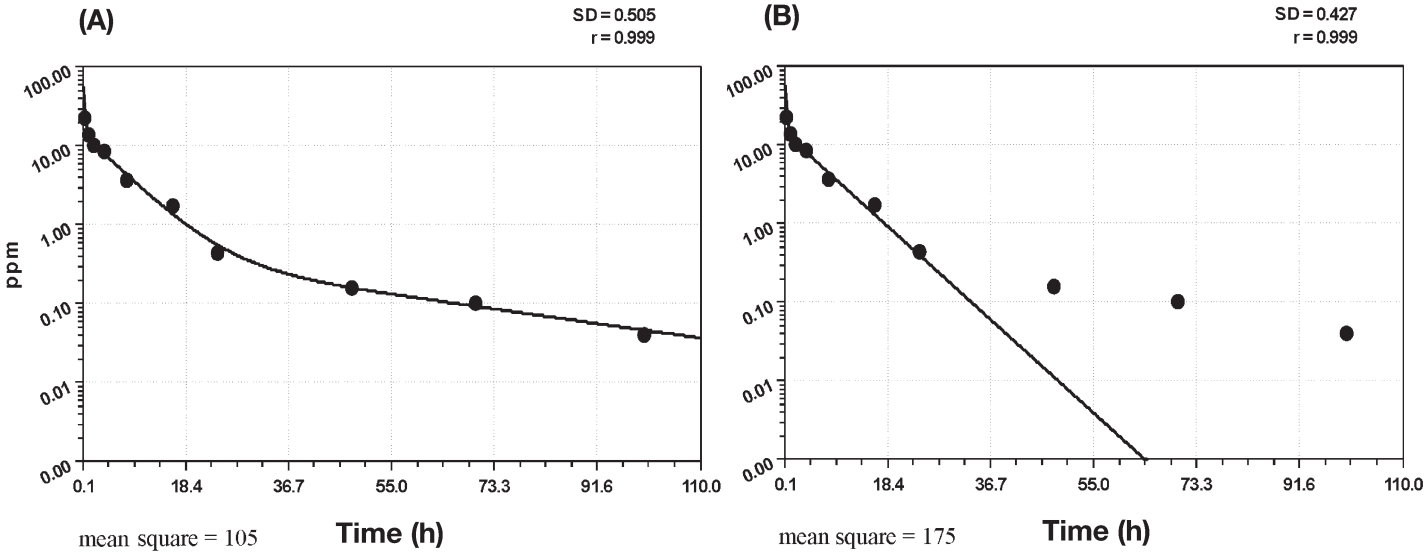

Fig. 1. *Sparus aurata.* Semi-log plot of flumequine plasma concentration vs time following injection against (A) a 3- or (B) a 2-compartment open pharma cokinetic model

Table 1. *Sparus aurata.* Plasma concentration (µg ml^{-1}) of flumequine (FLU) (mean ± SD) following a single intravascular injection (10 mg kg^{-1} fish) or oral administration (20 mg kg^{-1} fish) to gilthead sea bream (170 g; n = 4 to 5) held at 19°C. Time points: hours post-administration

Time points	Intravascular	Oral
0.5	22.71 ± 4.92	
1	14.09 ± 2.25	
2	10.36 ± 1.25	0.24 ± 0.06
4	8.45 ± 2.53	0.28 ± 0.03
8	3.64 ± 1.45	1.02 ± 0.22
16	1.72 ± 0.21	1.73 ± 0.34
24	0.43 ± 0.20	0.80 ± 0.06
48	0.16 ± 0.09	0.50 ± 0.01
70	0.10 ± 0.02	0.34 ± 0.02
100	0.04 ± 0.01	0.12 ± 0.02

were identified according to Mercedes & Blanch (1994) and Bakopoulos et al. (1995), respectively. Bacterial aliquots were taken from glycerol stocks and maintained on tryptic soya agar (TSA) supplemented with 2% NaCl. Single colonies were obtained from pure cultures (third passage on TSA), transferred to 10 ml tryptic soya broth (TSB) and incubated aerobically for 24 to 48 h at 22°C to a final cell density of approximately 10^6 cells ml^{-1} before being transferred to microtiter tray wells. Sterile tissue-culture plates (96-well, flat bottom) with lids were used. To each well, we added 50 µl MHB, 50 µl antibiotic solution and 10 µl of each bacterial culture (10^4 cells ml^{-1}) with a multipoint pipette (Eppendorf). Plates were incubated for 48 h at 22°C. Using a microplate reader (ASYS Hitech), each MIC was defined as the lowest drug concentration that inhibited bacterial growth. MIC testing was performed in triplicate. Individual MICs are the means of replicate results performed twice within a 1 wk period.

Table 2. *Sparus aurata.* Calculated pharmacokinetic parameters of FLU intravascularly injected (10 mg kg^{-1} fish) or orally administered (20 mg kg^{-1} fish) into gilthead sea bream (170 g) at 19°C. α, β, γ: slopes of monoexponential declining curves; $t_{1/2\alpha}$: distribution half-life of the drug; $t_{1/2\beta,\gamma}$: half-lives of the intermediate phase and the terminal elimination of the drug, respectively; $V_{d(ss)}$: apparent volume of distribution of the drug at steady-state; V_c: apparent volume of the central compartment; CL_T: total body clearance of the drug; $AUC_{0\rightarrow\infty}$: area under the drug concentration curve extrapolated to infinity; MRT: mean residence time; *F*: bioavailability

Parameter	Intravascular	Oral
α (h)	3.47	
β (h)	0.16	
γ (h)	0.023	
$t_{1/2\alpha}$ (h)	0.2	
$t_{1/2\beta}$ (h)	4	
$t_{1/2\gamma}$ (h)	30	
$V_{d(ss)}$ (l kg^{-1})	0.57	
V_c (l kg^{-1})	0.15	
$AUC_{0\rightarrow\infty}$ (µg h^{-1} ml^{-1})	119.59	70.10
CL_T (l kg^{-1} h^{-1})	0.05	
MRT (h)	11	
F (%)		29

RESULTS

Pharmacokinetics

The plasma levels of FLU from both routes of administration are presented in Table 1; the calculated pharmacokinetic parameters in Table 2. The distribution half-life ($t_{1/2\alpha}$) and the half-lives of the intermediate phase ($t_{1/2\beta}$) and the terminal phase of elimination ($t_{1/2\gamma}$) were calculated to be 0.2, 4 and 30 h, respectively. The apparent distribution volume of the drug at steady-state ($V_{d(ss)}$) and the apparent volume of the central compartment (V_c) were calculated to be 0.57 and 0.15 l kg^{-1}, respectively. The mean residence time (MRT) was estimated to be 11 h and the clearance of the drug (CL_T) was calculated to be 0.05 l kg^{-1} h^{-1}. The bioavailability (*F*%) of FLU was 29%. The maximum plasma concentration following oral administration was 1.73 µg ml^{-1} measured 16 h post-administration. No peaks indicating metabolites of FLU in the plasma were observed.

MICs

The MICs of the drug using distilled water supplemented with 2% NaCl against *Vibrio anguillarum* Serotype 1b, *Photobacterium damsela* ssp. *piscicida*, *V. alginolyticus*, *V. damsela* and *V. fluvialis* were 0.15, 0.3, 1.2, 0.019 and 0.15 µg ml^{-1}, respectively; however, the addition of 10 mM Ca^{2+} and 55 mM Mg^{2+} to the medium resulted in MICs of 4.78, > 38.25, 38.25, 0.15 and 4.78 µg ml^{-1}, respectively (Table 3).

Table 3. Minimum inhibitory concentrations (MICs) (µg ml^{-1}) of FLU using either distilled water with added 2% NaCl or additionally supplemented with 10 mM Ca^{2+} and 55 mM Mg^{2+} against important bacterial pathogens in Mediterranean mariculture at 22°C. API: Analytical Profile Index

Bacterial pathogens	API 20E value	MIC values 2% NaCl	MIC values + cations
Vibrio anguillarum 1b	3247524	0.15	4.78
Photobacterium damsela ssp. *piscicida*	2005004	0.3	>38.25
V. alginolyticus	4247525	1.2	38.25
V. damsela	2015004	0.019	0.15
V. fluvialis	3247127	0.15	4.78

DISCUSSION

The present study evaluates FLU as a treatment agent in Mediterranean mariculture, investigates its pharmacokinetics in the main species reared in euryhaline fish farming (the gilthead sea bream *Sparus aurata*) and determines the MIC against important bacterial pathogens involved in this mariculture industry.

The distribution half-life ($t_{1/2\alpha}$) of FLU following intravascular injection into gilthead sea bream (0.2 h) indicates rapid distribution from the blood to the tissue compartments of the fish. Longer $t_{1/2\alpha}$ of the drug have been estimated for the sea bass *Dicentrarchus labrax* (1.05 h: Rigos et al. 2002).

The bioavailability (F%) of FLU following oral administration to gilthead sea bream was relatively low (29%), but close to the values reported for other farmed fishes. The F of FLU has been calculated to be 44% in channel catfish (Plakas et al. 2000) and in the range of 31 to 59% in coldwater fishes (Rogstad et al. 1993, Elema et al. 1995, Martinsen & Horsberg 1995, Samuelsen & Ervik 1997, Hansen & Horsberg 1999).

First-pass effect and malabsorption were previously suggested as factors limiting FLU bioavailability in freshwater fishes (Elema et al. 1995). FLU is a weak acid and, consequently, its absorption may be reduced by the unfavourable alkaline intestinal environment of marine fish. The possible complexing of FLU with cations such as Ca^{2+} and Mg^{2+} in the feed and in the marine environment could also partially explain the low F in marine fishes (Samuelsen & Ervik 1997). Thus, a further reduction in bioavailability is likely to occur during at-site administrations of FLU through the diet, since the drug is given to gilthead sea bream in aqueous suspension.

The half-life of the terminal phase of elimination ($t_{1/2\gamma}$) of FLU in gilthead sea bream (30 h) was slower than that reported for other farmed fishes kept at high water temperatures. The elimination half-life of FLU was reported to be 10 h in sea bass kept at 18°C (Rigos et al. 2002) and 22 h in channel catfish maintained at 24°C (Plakas et al. 2000). The inter-specific differences observed in the elimination of FLU can be due to the fact that in fishes, as in mammals, species-dependent differences exist in the liver microsomal cytochrome P450-dependent mixed-function oxidase system which is the primary mechanism in catalysing the oxidative metabolism of a variety of drugs, together with other exogenous compounds (Stegeman 1989).

The apparent distribution volume of the drug at steady-state in gilthead sea bream ($V_{d(ss)}$ = 0.57 l kg^{-1}) is similar to that in channel catfish (0.527 l kg^{-1}) (Plakas et al. 2000), but smaller than in sea bass (1.51 l kg^{-1}: Rigos et al. 2002). The apparent volume of the central compartment (V_c = 0.15 l kg^{-1}) of FLU in gilthead sea bream is also smaller compared than that in sea bass (0.63 l kg^{-1}: Rigos et al. 2002), indicating a low distribution of FLU from the blood to the tissue compartments of gilthead sea bream.

The total clearance of the drug (CL_T) in gilthead sea bream is identical to that reported for Atlantic halibut (0.05 l kg^{-1} h^{-1}: Samuelsen & Ervik 1997). A lower CL_T has been calculated for channel catfish (0.0149 l kg^{-1} h^{-1}: Plakas et al. 2000). In contrast, higher values of CL_T have been reported for sea bass (0.156 l kg^{-1} h^{-1}: Rigos et al. 2002).

Following oral administration (20 mg kg^{-1}) of FLU to gilthead sea bream, the maximum plasma concentration was relatively low (1.73 µg ml^{-1}). In comparison, the maximum plasma concentration (C_{max}) of FLU was 3.1 µg ml^{-1} at a dose of 5 mg kg^{-1} in channel catfish (Plakas et al. 2000), 1.4 to 2.7 µg ml^{-1} at a dose of 10 to 25 mg kg^{-1} in Atlantic halibut (Samuelsen & Ervik 1997, Hansen & Horsberg 1999), 1.07 to 1.91 µg ml^{-1} at a dose of 5 mg kg^{-1} in rainbow trout (Sohlberg et al. 1990, 1994), 1.42 to 2.26 µg ml^{-1} at a dose of 25 to 50 mg kg^{-1} in Atlantic salmon (Rogstad et al. 1993, Martinsen & Horsberg 1995), and 1.9 µg ml^{-1} at a dose of 10 mg kg^{-1} in turbot (Hansen & Horsberg 1999).The time of FLU to reach its maximum plasma concentration in gilthead sea bream (16 h) is within the values reported by the aforementioned studies (6 to 54 h).

Based on the findings of Tsoumas et al. (1989), a bacterial pathogen is deemed as 'resistant', 'moderately susceptible' and 'susceptible' to oxolinic acid when its MIC is >1.0, 0.125 to 0.5 and <0.0625 µg ml^{-1}, respectively. MICs reflect a quantitative laboratory measurement of bacterial sensitivity to tested drugs without providing a complete picture of the *in vivo* situation. In addition, combining drug tissue levels with MIC data to assess the clinical significance of drugs may complicate matters still further, since such drug concentrations are calculated by laboratory protocols and may not represent the biological activity of the drug in the target organism (Smith et al. 1994). However, a recent study demonstrated that it is valid to predict the treatment efficacy of oxytetracycline from *in vitro* data (Bruun et al. 2003). A rather theoretical assessment of a drug's efficacy against bacterial pathogens is the presumption that it is effective when its maximum plasma concentration following administration to the target species exceeds a factor of 4:1 (peak plasma level:MIC; Stamm 1989). However, such assessment has been considerably criticised (Smith et al. 1994) and should be treated with caution.

According to guidelines of Tsoumas et al. (1989), our MICs for FLU using water supplemented with 2% NaCl (1.2 to 0.019 µg ml^{-1}) indicate that only *Vibrio alginolyticus* is resistant to FLU (MIC >1 µg ml^{-1}), with *Photobacterium damsela* ssp. *piscicida* and *V. fluvialis*

showing a moderate susceptibility (0.15 to 0.3 µg ml^{-1}). The most sensitive bacteria to FLU were *V. damsela* (MIC = 0.019 µg ml^{-1}) and *V. anguillarum* Serotype 1b (MIC = 0.15 µg ml^{-1}); the latter is the most pathogenic and commonly occurring bacterial pathogen in euryhaline fish farming. However, the addition of 10 mM Ca^{2+} and 55 mM Mg^{2+} to the medium (approximating Mediterranean salinity in these cations) had a dramatic effect on FLU activity, increasing its MIC 30 times for *V. anguillarum*, *V. alginolyticus* and *V. fluvialis* and 8 times for *V. damsela*. In addition, no inhibition of *P. damsela* ssp. *piscicida* growth occurred at the highest drug concentration used (38.25 µg ml^{-1}) when these 2 cations were added. These findings were not unexpected, since previous studies have stressed the strong antagonism of several drugs by marine cations (Smith 1989, Lunestad & Goksoyr 1990, Barnes et al. 1995, Pursell et al. 1995, Torkildsen et al. 2000). The mechanism by which marine cations influence the antibacterial activity of drugs it not well known. Some assumptions include the formation of complexes by chemical interactions, decreased permeability of the bacterial cell by neutralisation, or interaction with the medium itself (Torkildsen et al. 2000, Lunestad & Samuelsen 2001). The relative importance of these factors for bacterial pathogens and antibacterial agents of interest requires further investigation. The location at which the inhibitory effect of the cations on the drug is induced has also not been documented. It is likely that inhibition takes place mainly during the absorption stage.

The maximum plasma concentration of FLU following oral administration to gilthead sea bream (1.73 µg ml^{-1}) exceeds a factor of 10:1 (peak plasma level:MIC) for the MICs obtained for distilled water with added 2% NaCl for *Vibrio anguillarum* Serotype 1b, *V. damsela* and *V. fluvialis*, being almost 6 times higher than the MIC for *Photobacterium damsela* ssp. *piscicida*. Stamm (1989) reported that the effect of FLU is uncertain only against *V. alginolyticus*. However, considering the MICs for the medium supplemented with cations, it can be concluded that FLU is not effective against any of the bacterial pathogens tested. Further investigation including challenge tests with these bacterial pathogens followed by FLU treatment is required to confirm these findings.

In conclusion, this study demonstrates an adequate kinetic profile of FLU in gilthead sea bream characterised by rapid distribution and elimination and low tissue penetration. The bioavailability of the drug is comparable with that for other farmed fishes. The reported MICs of FLU indicate that the presence of marine cations in the medium have a significant impact on the activity of the drug, rendering questionable its use against bacterial pathogens in Mediterranean fish farming.

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