

SESSION II: Pharmacologic Research in Aquaculture

Drug Disposition in Fish

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INTRODUCTION

Fish are intimately linked to the water environment in which they live. They breathe, ion regulate, osmoregulate, thermally cue, urinate, defecate, feed, attain buoyancy, reproduce and develop propulsion in, and in response to, the water media. Water is a universal solvent and acts as a contiguous common pool for fish, to which substances are eliminated or extracted from. This common modality provides for an array of interactions between environmental, piscine and chemical factors.

Drug absorption and disposition in fish is highly dependent upon their specialized physiology and their close relationship with the aquatic environment. In many regards, water is an outward extension of the fluid space of the aquatic animal. Similar types of physiochemical and kinetic interactions as seen with drugs within the animal exist between water and the dissolved constituents in the water as well as between the animal and the environment. The interactions at each of these three levels are important determinants of drug fate. The relative importance of each is dependent upon the characteristics of the drug, characteristics of the interactive compartments and the route of exposure.

Physiological responses by the fish to environmental factors also play a role in drug disposition. These environmental stressors and responses are common in the aquatic environment, especially in intensive aquacultural settings. Such effectors, along with the fishes unique physiology and interaction with water, provide the backdrop for drug disposition in fish.

PHYSIOLOGY

Fish maintain a number of unique physiological adaptations which are germane to drug disposition. Some of these adaptations are a direct outgrowth of life in the aquatic environment. Gills, for example, while instrumental in gas exchange, nitrogenous waste elimination, acid-base balance and ion regulation, also function in drug absorption and disposition. Much like alterations available in the lungs, the oxygen exchange capabilities

of the gill can be altered by changing water and blood flow rates as well as surface area. In the case of the gill, blood and water are in an oppositional or counter current flow pattern. This pattern, while optimizing oxygen extraction capacity, also may facilitate efficient drug uptake from water and elimination from the animal. The relative importance and direction of these processes are dependent upon the chemical's partitioning characteristics, route of exposure and physiological determinants. Environment alterations in dissolved oxygen content can have major effects upon ventilation rates and extent of perfused surface area. These parameters secondarily alter drug disposition in affected species.

Osmoregulation is an extremely important process for aquatic species. Freshwater fish, being hyperosmotic to the aquatic environment, are in a continual battle to remove excess water. In large measure, osmoregulation in freshwater fish is a function of the kidney. However, unlike mammals, the freshwater teleost kidney plays little role in excretion of nitrogenous waste. Urine in these animals is essentially water, with a specific gravity of approximately 1.001; nevertheless, urine is produced in copious amounts, approximately twice that of mammals. Conversely, marine species, being hypoosmotic to saltwater, are essentially living in a desiccative environment. Urine is, at best, only sparingly formed by many marine fishes. To facilitate these functional responses, large differences in regard to nephron structure exist among divergent fish species. These features are influential in determining the renal contribution to xenobiotic disposition.

Xenobiotics have been demonstrated to be eliminated in the urine of a variety of fish species. Glucuronide, sulphate, and taurine conjugates are known to be excreted in a carrier mediated fashion by the fish kidney. The qualitative and quantitative contribution of renal excretion to the total elimination of drugs from fish has only been delineated for a few compounds. In select circumstances, where comparable data is available, fish appear to be less dependent on renal elimination than their mammalian counterparts. More studies are necessary to extend and confirm this generalization.

Fish are extremely efficient biliary concentrators of xenobiotics. Much like their mammalian counterparts, xenobiotic molecular weight and polarity criteria appear to be operative for excretion of drugs into the bile of fishes. Bile formation rates ranging as low as 1/50th of that of mammals and slow bile elaboration rates appear to be major determinants of fishes concentrating abilities. Bile accumulation during periods of fasting associated with fish morbidity may further influence drug disposition by delaying intestinal elaboration of compound-containing bile. Enterohepatic recirculation of both endogenous and exogenous compounds has been shown to occur in fish. Due to the high xenobiotic concentrations in bile and delayed elaboration, these conditions, in conjunction with enterohepatic recirculation, may prove to influence the apparent residence time of drug residues.

A number of blood profile characteristics influential in drug disposition differ significantly between aquacultural species and mammals. While the data base is limited to the relatively few species examined, plasma protein content appears to be significantly lower in fish. Likewise, binding of xenobiotics to plasma proteins is also comparatively low in fish. To date, a significant number of drugs in fish exhibit binding which is nonsaturable and nonspecific within therapeutically relevant concentrations. This is contrasted by

saturability and specificity for the same compounds in mammals. In both groups, the importance of plasma protein binding resides in its limiting effect upon drug distribution and elimination.

Blood pH also differs between mammals and fish. For example, blood pH values ranging from 7.5 to 8.0 have been reported for trout. While pH values average approximately 8.0 under normal conditions, if stressed by handling values drop to around 7.5, presumably due to an ensuing acidosis. Dependent upon the pKa of the drug, the high pH of fish blood under normal conditions may alter the degree of drug ionization as compared to mammals.

Temperature has a pervasive influence on all aspects of fish physiology. Fish of the same species acclimated at largely disparate temperatures are essentially different animals. Everything from muscle fibre activity to lipid composition are altered with temperature. Drug absorption, metabolism and disposition are also subject to temperature modulating effects. A number of studies with antibiotics have identified that, as compared to warm acclimated animals, cold acclimated animals retain higher levels of drugs for longer periods of time. Recent work in our laboratory, as well as by others, has shown that temperature related residue retention may be compound specific. Oxolinic acid and enrofloxacin, for example, have shown equivocal relationships of residue residence with temperature. Conversely, pharmacokinetic modelling of oxolinic acid in catfish has shown that, with a 10° C temperature drop, significant reductions in the total body clearance, as well as a resultant lengthening of the $t_{1/2\beta}$ were evident. Other concerns regarding temperature and drug residues appear to be emerging. Studies in catfish with the folic acid antagonist ormetoprim indicate that at least for this compound, changes in temperature may result in differing metabolite profiles.

BIOTRANSFORMATION

Fish are capable of a wide variety of the biotransformation reactions observed for mammals. Although the scope of reactions is similar, differences do exist between fish and mammals in the metabolic handling of chemicals. Among these are differences in reaction rates, the relative contribution of a given pathway and the products formed. In addition, among fish, large species differences exist in both the phase I nonsynthetic and the phase II conjugative pathways. The implications of biotransformation for excretion, pharmacodynamics and toxicity are the same as for mammals.

Like mammals, cytochrome P450 in fish consists of multiple isozymic forms. These isozymes, while offering polysubstrate capabilities, do exhibit some specificity in product formation and response to inducers. The P450 system in fish, as compared to mammals, includes a selective response to inducers, ideal temperature compensation, lower temperature optima, and lower P450 dependent specific activities.

Other phase I metabolic pathways such as hydrolytic and reductive reactions have been demonstrated in fish. These enzymes have not received as much attention as the P450 mediated oxidative reactions, however, considerable information is present for specific substrates. Hydrolysis reactions have been demonstrated for substrates as diverse as pesticides and the fish anesthetic tricaine methane sulphonate (MS-222).

The most important phase II reactions are glycosylation, sulphation, mercapturic acid formation, amino acid conjugation, and acetylation. *In vitro* studies have shown that UDP-glucuronosyltransferase (UDPGT) in fish liver processes many of the same properties as mammalian enzymes. In fish, as in mammals, the enzyme exists in multiple forms, appears in the microsomal fraction, and exhibits induction with beta-naphthoflavone. UDPGT activities among fishes vary widely. Additionally, UDPGT activities in fish vary above and below those of mammals, dependent upon the substrate and fish species. Optimal conditions of pH and temperature for the UDPGT enzyme vary considerably with fish species.

Amino acid conjugation has been identified in fish as a route utilized for conjugation of carboxylic acids. In contrast to mammals, where glycine is the primary amino acid conjugate, taurine is currently the only amino acid conjugate rigorously identified in fish. These conjugates have been isolated primarily for marine species.

Sulphate conjugation has been reported in a variety of fish species for compounds which contain aliphatic and aromatic hydroxyl groups. Sulphate conjugation appears to be cofactor limited and quantitatively very species dependent. Comparatively, fish demonstrate less sulphotransferase activity than most common laboratory mammals.

Glutathione, mercapturic acid and other intermediate products of this enzymatic cascade have been found in bile, urine and excreta of a wide variety of fish. Glutathione S transferase, the enzyme responsible for the formation of the glutathione conjugate has been purified from a variety of aquatic species with multiple forms of the enzyme isolated. While large differences in specific activities exist among fish species, activities generally are similar to those of mammals. The capacity of glutathione conjugation can be exceeded and depleted, as in mammals, resulting in toxicity of reactive compounds.

Acetylation is a common route of biotransformation in fish. Sulphonamides, tricaine methane sulphonate, 2-aminofluorene, and nitroanisole are well documented substrates. Comparison of acetylation activities in fish, with mammals such as the rabbit, indicate lower activities than mammals.

WATER EXPOSURE

Fish exposed to drugs via the water may demonstrate branchial, dermal and gastrointestinal uptake. Many factors including abiotic and biotic water quality parameters, compound solubility in water and also solubility in biological membranes are operative determinants of drug uptake from water. Compounds which exhibit appreciable water solubility and moderate lipophilicity are readily transported into the animal. Compounds which have a high lipophilicity and low water solubility tend in natural waters to adhere to colloidal organics and to surfaces, thus precluding any meaningful bioavailability to the animal. Compounds of high molecular weight with high water solubility and low lipophilicity will be only marginally absorbed through the biological membranes encountered at the gill and other surfaces.

Water quality parameters may alter chemical form and availability of drugs. As within the organism, environmental pH is instrumental in determining drug movement and form. The pH of natural waters ranges from 4 to 9, with drug carrier vehicles and acid rain, as well

as other factors extending that range. The influence of these exogenous factors is dependent upon the buffering capacity of the water (referred to as alkalinity by limnologists). Compound ionization/nonionization ratios, determined in large part by the water pH, dictate both the water solubility and the membrane solubility.

ORAL ADMINISTRATION

Many of the drugs currently used in conjunction with fish are administered orally. As described elsewhere in this discussion, chemical form, dose and temperature are important factors influencing bioavailability of drugs in fish. Other features, common both to fish and mammals, which are instrumental to bioavailability include the nature of the matrix, gastrointestinal clearance times, and drug ionization.

Considerable variations in drug bioavailability occur with different drugs. Values ranging from 6 to 100% have been reported for a variety of antimicrobial drugs in fish. There is currently no clear relationship of bioavailability to species.

PHARMACOKINETICS

A number of similarities and differences exist between mammals and fish in regard to the pharmacokinetics of drugs. While new information and additional studies with other drugs may modify these generalizations, at present a number of statements may be made. Where the data base allows comparison, fish generally exhibit longer half lives, larger volumes of distribution and lower clearances than their mammalian counterparts. These comments, of course, as discussed earlier are dependent upon acclimation temperature and probably subject to exceptions. For the few cases where examination is feasible, the pharmacokinetic interrelationships of drugs in mammals appear to be preserved upon extrapolation to fish. That is to say, that while the absolute values and proportions vary, the kinetic interrelationships of similar drugs to each other in mammals grossly extrapolate to fish handled under comparable experimental conditions of dosage and sampling.

Pharmacokinetic similarities and differences also exist between fish species. Studies with oxolinic acid in trout and catfish, for example, indicate that there are both species and temperature related differences in the kinetics of drugs. Comparison of catfish and trout at a similar temperature (14° C) or at approximately their preferred temperatures (trout 14° C; catfish 24° C) indicate the two species were different independent of acclimation temperature and because of acclimation temperature. Clearance values of the two species, for example, were more similar at their preferred temperatures than when held at a common temperature (14° C). In contrast, volume of distribution, while different between the two species, did not vary with temperature. Oral bioavailability also appears to have a temperature component; however, such varying results have been reported as to preclude a generalized statement regarding bioavailability and temperature at this time.

Other pharmacokinetic findings may bear some relevance to drug development and use in fish. At least two drugs have demonstrated extended half lives upon multiple dosing of fish. This is presumably related to saturation of some step in the excretory process. Bioavailability for oral administration, as in mammals, is dependent in part upon the

chemical form of the drug. Similarly, relative bioavailability appears to be marginally dependent on dose with decreases in bioavailability observed with increasing dose. All of these processes appear to require attention when dealing with drugs in fish.

It is apparent that the manner in which kinetic studies are performed can make significant differences in the kinetics observed. This not only includes temperature, water quality (especially in cases of water exposures) and acclimation duration, but also how the animals were sampled (removed from the water versus cannulated; free swimming versus restrained; volumes of samples relative to the size and environment of the animal). Published, preliminary and anecdotal information suggests that all of these factors may be significant determinants of pharmacokinetics in fish.

SUMMARY

Drug disposition in fish shares many similarities with mammals and presents a number of unique differences. In few other situations does the environment in which an animal resides play such a critical role in the fate of drugs. Temperature and water quality considerations (pH, hardness, alkalinity, dissolved oxygen) set the stage physiologically for the dynamics of the drug interaction. Other parameters such as the drug solubility within water and the fish as well as constant exposure to a common medium, present concerns which are not an issue in mammalian studies. Physiologically, the intimate link of fish with the environment presents avenues either nonexistent or of minor consequence in the fate of most drugs in mammals. Fish adept in the processing of xenobiotic compounds provide an interesting adaptive approach to drug disposition.