COMPARATIVE PLASMA PROTEIN BINDING OF ORMETOPRIM IN MARINE AND FRESHWATER FISH SPECIES

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Plasma protein binding can be an important determinant in the pharmacokinetics of drugs and other xenobiotic compounds. Such binding may influence the volume of distribution as well as the clearance of compounds from the body. Recent studies with a variety of antibacterial agents in freshwater teleosts have demonstrated differences and similarities to mammals in regards to the saturability and degree of protein binding (Plakas et al, Xenobiotica 18:83, 1988.; Squibb et al. Vet and Human Toxicol. 30:31, 1988). No such comparable information is available for cross comparisons with salt water fish species. The objective of this study was to extend the scope of observations regarding protein binding from freshwater species to include species representative of both marine cartilaginous and bony fishes. For this purpose ^{14}C ormetoprim, a dihydrofolate reductase inhibitor, was utilized as the model compound.

Male and female flounder (Pseudopleuronectes americanus), sculpin (Myoxocephalus octodecimspinosus), dogfish shark (Squalus acanthias), skate (Raja erinacea) and catfish (Ictalurus punctatus) were utilized for the experiments. Blood samples were collected from 3 individuals of each species by caudal peduncular or cardinal sinus venipuncture. Four vials containing unlabeled and radiolabeled ormetoprim at either 0.04, 0.30, 1.0, or 3.0 mM were prepared for receipt of plasma from each fish. The vials containing plasma and ormetoprim were vortexed, then incubated at the fishes acclimation temperature for 30 min. Following incubation, total radioactivity was sampled for each concentration, and the remainder subjected to micropartition filtration at a molecular weight cut off of 22,000 - 30,000 (Centrifree, Micropartition Systems - Amicon Corp. Danvers, MA). Free drug was determined by liquid scintillation counting of the ultrafiltrate. Plasma protein binding was determined as the difference between the total radioactivity and that present in the ultrafiltrate. Absorption to the partition apparatus was independently determined with the pre and post filtration technique using ormetoprim spiked protein free filtrate. This value was subtracted from calculated bound values for the experimental samples. All protein binding values were normalized mathematically in regards to protein content. Plasma protein levels were measured as described by (Lowry et al J. Biol. Chem. 193:265, 1951). The results are shown in Table 1.

These data clearly demonstrated that plasma protein binding of ormetoprim varies considerably in extent and saturability over the concentration range and species examined. While the degree of protein binding in freshwater teleosts (trout, catfish) differed widely, binding was clearly non-saturable for both species. In contrast the marine teleosts (flounder, sculpin) were similar to each other in terms of the extent of binding, but the flounder exhibited saturability over a narrow concentration range. Binding and saturability data for the shark and skate demonstrated no apparent trends. This may have been related to a predelection of unknown cause to plasma clotting in elasmobranchs. Anticoagulants were not used due to known influences of such agents upon protein binding.

The results presented for ormetoprim suggests that extrapolation of binding data from one fish species to another is inappropriate. The

differences observed may be related to electrostatic, lipophilic or receptor interactions specific to each fish species rather than specific groups of fish. Nonspecific binding as evidenced by little or no change in bound drug profiles with increasing concentration appears to be the predominant pattern for the fish examined. This may be the result of partition coefficient dependent lipid binding (Schmieder and Henry, Comp. Biochem. Physiol. 91C: 413, 1988) or limitations in the range of ormetoprim concentrations examined. Further studies are required to identify the mechanisms for the observed differences. (Supported by Investigators and FDA-000158-01).

Table 1. Percent of different ormetoprim concentrations bound in vitro to fish plasma proteins

Species	Acclimation Temperature	Ormetoprim mM			
	°C	0.04	0.30	1.0	3.0
Flounder	11-13	24.6±5.3*	18.7±4.4	9.9±2.0	7.2±0.98
Sculpin	11-13	17.5±5.1	14.2±7.3	13.3±12.2	9.8±3.7
Catfish	23-24	8.3±3.5	7.0±7.2	7.2±6.0	8.6±5.9
Skate	11-13	12.4±5.4	8.9±10.4	8.7±6.8	17.1±7.1
Shark	11-13	0.08±0.14	ND	3.3±5.7	3.1±5.4
Trout+	13-14	31.6	33.9	28.6	28.8

*Mean ± SD (N = 3)
+pooled plasma samples (N = 3)
ND None detected