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A REVIEW OF FISH MODEL IN EXPERIMENTAL PHARMACOLOGY

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ABSTRACT
Recognizing differences in the physiology of fish is essential to understanding the problems associated with drug approval for the aquatic environment. A wide variety of experiments and the use of drugs in fish have been elucidated in various literatures. Antibacterial, antiparasitic and anaesthetic drugs, besides the pharmacokinetic and pharmacodynamic parameters have been well experimented on the fish. Several groups of drugs, such as tetracyclines, penicillins, macrolides, quinolones, sulfonamides, immunostimulants, anticancer agents, herbal drugs and vaccines, have been successfully experimented or used in fish. Hence, fish is also used as a new model organism for different experimental studies of pharmacology and toxicology. The potential for the application of research findings to both human and environmental health issues makes fish species attractive and valuable alternative models in various diseases, including cancer, and pharmacological and toxicological research. Zebrafish has emerged as a major model organism in the developmental genetics, neurophysiology or biomedical research.
KEYWORDS: Drugs, experimental studies, fish as model, pharmacology and toxicology.

INTRODUCTION

The world of fish pharmacology is now changing quickly. As aquaculture continues to expand, there is a need for greater knowledge of medicinal treatments both for the prevention and treatment of diseases, and for the economic husbandry of fish. A wide variety of experiments and the use of drugs in fish have been elucidated in various literatures. Now, it has been considered that aquaculture is a rapidly emerging industry. Recognizing differences in the physiology of fish is essential to understanding the problems associated with drug approval for the aquatic environment. Ectothermic animals change body temperatures through a wide range, affecting both the uptake and distribution and biotransformation of drugs. Antibacterial, antiparasitic and anaesthetic drugs, besides the pharmacokinetic and pharmacodynamic parameters have been well experimented on the fish. Several groups of drugs, such as tetracyclines, penicillins, macrolides, quinolones, sulfonamides, immunostimulants, anticancer agents, herbal drugs and vaccines, have been successfully experimented or used in different fish species. It would have been useful to do more pharmacological experiments in fish on emerging new drugs. It will be nice to observe the effects of breeding induction agents, immunostimulants, anticancer agents, herbal drugs and vaccines, all used in fish husbandry and therapeutic strategies in aquaculture.

In pharmacological and toxicological research, fish has also been used as a new model organism to create the experimental carcinogenesis or cancer and ultimately to evaluate the anticancer activity of drugs. The potential for the application of research findings to both human and environmental health issues makes fish species attractive and valuable alternative models in the carcinogenesis and toxicity research. The zebrafish (*Danio rerio;* Syn. *Brachydanio rerio*), a freshwater tropical fish, can be kept easily and cheaply in large numbers in laboratory, where it breeds all year round. Therefore, the zebrafish has been recognized as a suitable model for different experimental studies[1]. Cell lines provide an important biological tool for carrying out investigations into physiology, virology, toxicology, carcinogenesis and transgenics. Teleost fish cell lines have been developed from a broad range of tissues such as ovary, fin, swim bladder, heart, spleen, liver, eye muscle, vertebrae, brain and skin. One hundred and twenty-four new fish cell lines from different fish species ranging from grouper to eel have been reported. Presently, about 283 cell lines have been established from finfish around the world[2].

It is the time when this review article will better solve the problems or understanding about the choice and use of the fish as models in experimental pharmacology and toxicology. This article allows accessible information to the researchers, pharmacy scientists, veterinarians, as well as to students who are not familiar with the complexities of fish pharmacology and toxicology.

FISH AS EXPERIMENTAL MODEL ORGANISM

Fish has now been considered as the model organism for conducting different experimental studies, including those of pharmacology and toxicology. The mangrove-dwelling fish, *Rivulus marmoratus*, is the only vertebrate that is a synchronous, internally self-fertilizing hermaphrodite. This unique reproductive mode yields offspring with little genetic variation, which offers significant advantages for the use of this species in bioassays[3]. In the 1960s to mid-1970s, the fish species used as models for carcinogenesis studies were primarily the zebrafish (*D. rerio*) and the guppy (*Poecilia reticulata*). Species that have predominated in later years can be divided into two groups: the larger fish, e.g., rainbow trout, and the small aquarium fish, including rivulus (*R. marmoratus*), guppy, sheepshead minnow (*Cyprinodon variegatus*) and medaka (*Oryzias latipes*). The contaminations associated neoplasia, including aflatoxin-induced hepatocellular carcinoma (HCC) in rainbow trout (*Onchorynchus mykiss*) fish, have also led to the study of fish as alternative models in carcinogenesis and toxicity bioassays[1].

In fact, the zebrafish has been reported[4] to be an important model organism in the developmental genetics, neurophysiology and biomedicine. Over 400 labs worldwide now routinely use the zebrafish in several researches, and there is an increasing interest in its use as a model for understanding the genetic basis of behaviour[5]. The zebrafish is increasingly important in biomedical research, particularly as a model of human disease and for the screening of therapeutic drugs[6]. Hence, the zebrafish has emerged as a major model organism for biomedical research[7], and it is a premiere model organism to study the vertebrate development. The zebrafish may also be a powerful model for the study of human diseases because many cellular processes are conserved throughout vertebrate evolution, including corresponding disease genes[8]. Tumours in zebrafish can be generated by treatment with chemical carcinogens or by genetic approaches. Liver has been the chief target organ for tumorigenesis after carcinogen treatment while many other tissue-specific tumours have been generated by tissue-specific expression of proven oncogenes. A remarkable similarity has been demonstrated in the molecular hallmarks during liver tumorigenesis between humans and zebrafish, thus validating the zebrafish model

for human cancer studies. The zebrafish models for liver cancers have been successfully established which are now increasingly used as a promising animal model for cancer research. Hence, these models will be characterized in order to understand the molecular and genetic mechanisms of liver carcinogenesis as well as for anticancer drug discovery[9].

SOME PHARMACOLOGICAL AND TOXICOLOGICAL *IN VIVO* STUDIES ON FISH

The innervation of the heart of the trout (*Salmo trutta* and *S. iredius*) was studied[10] with fluorescent histochemical and physiological methods. The catecholamine-containing nerves were revealed by the histochemical technique in the sinus venosus, atrium and ventricle. Stimulation of the vagus nerve or the ductus Cuvieri (especially at high-pulse frequency and duration) produced inhibition of the heart, which was blocked by atropine. After atropine treatment, an excitatory response was revealed which was blocked by guanethidine, bretylium or pronethalol. Finally, it has been suggested that the cardiac branch of the vagus nerve contains both inhibitory cholinergic and excitatory adrenergic nerves. The release of endogenous dopamine from teleost retinae was studied using high-performance-liquid-chromatography and electrochemical detection. The light evoked release of dopamine was inhibited by GABA and l-glutamate, whilst antagonists of these retinal transmitters stimulate release in the dark[11]. Aminergic metabolism was studied in discrete brain regions of the post-ovulated female rainbow trout using a liquid chromatography electrochemical detection method. 3-methoxytyramine (3-MT) was the major dopaminergic catabolite, suggesting that catechol-o-methyl transferase is the main dopamine (DA) catabolic enzyme. Two populations of brain regions were found: one with a high DA content and low 3-MT/DA ratio (hypothalamus and telencephalon), suggesting that these regions could present a high density of DA perikarya; the other with a high 3-MT/DA ratio (pituitary, preoptic area, myelencephalon and optic tectum), suggesting that these regions could present a high density of DA axonal endings. 5-hydroxytryptamine (5-HT) content differed, but a homogeneous distribution of monoamine oxidase was found in different brain regions. High 5-HT content was found in the hypothalamus and telencephalon[12].

The effect of salinity on the acute toxicity of cadmium was studied[3] in the tropical, estuarine, hermaphroditic fish, *R. marmoratus*. Live fibre bundles were isolated from the fast myotomal muscle of short-horned sculpin (*Myoxocephalus scorpius* L.) marine fish, and the isometric contractile properties and force-velocity relationship were determined at 5, 10 and 15°C. The maximum contraction speed of muscle fibres at 15°C was 2.4 times higher in 15°C- than in 5°C-acclimated fish. It was concluded that acclimation modifies the contractile properties of fast muscle fibres at both low and high temperatures[13]. Another important experiment which was conducted in fish is the study of molecular evolution of opioid receptor family. The cDNAs that encode 6 distinct opioid receptor-like proteins were isolated from the teleost fish, *Catostomus commersoni*. One of these encodes a 383-amino acid protein that exhibits greatest sequence similarity to mammalian opioid (m-opioid) receptors; the corresponding gene is expressed predominantly in brain and pituitary. Transfection of the teleost cDNA into HEK 293 cells resulted in the appearance of a receptor having high affinity for the m-selective agonist and for the nonselective antagonist naloxone. The data also indicated that the m-opioid receptor arose very early in evolution, perhaps before the appearance of vertebrates, and that the pharmacological and functional properties of this receptor have been conserved over a period of 400 million years implying that it fulfils an important physiological role[14]. Various drugs were evaluated[15] as regards the efficacy for the treatment of *Hexamita salmonis* infection in rainbow trout. The results confirmed the efficacy of nitroimidazoles: infection was completely eradicated not only by metronidazole (which has been recommended for hexamtosis treatment), but also by benznidazole, ronidazole and secnidazole. The non-nitroimidazoles albendazole, amnosidine, hethylcarbamazine and nitroscanate also completely eliminated the infection.

Venomous creatures have been the source of much recent research in the effort to find novel physiological tools and pharmaceuticals. Thus, an experiment was successfully performed by in fish (stonefish), suggesting that both functionally in experimental models and in western immunoblotting analysis, piscine venoms may possess structural as well as functional similarities as compared to the venoms of other species. The most potent effects of piscine venoms are on the cardiovascular system[16]. An experiment on fishes was conducted for the pharmacological characterization of melanocortin (MC) receptors suggesting the role for ACTH. The MC receptor subtypes have distinctive characteristic binding profiles. The MC4 receptors of trout and fugu fishes had similar affinity for α-MSH and β-MSH, and a much higher affinity for ACTH than does the human MC4 receptor. The fugu MC1, and the trout and fugu MC5 receptors also had higher affinity for ACTH-derived peptides than α-, β-, or γ-MSH. The ACTH-derived peptides might have played an important role at the MC receptors, while the specificity of the different subtypes for α-, β-, and γ-MSH peptides might have appeared at later stages during vertebrate evolution[17]. On the premise that human medicines may potentially induce similar pharmacological and toxicological profiles in fish and other lower vertebrates, a comparative approach was applied to β-adrenergic receptor antagonists (β-blockers) which are widely detected in surface water. The activities of these compounds may be observed more efficiently in fish than in mammals. Extensive mammalian pharmacological and toxicological studies are central to development of medicines and these can provide valuable information to guide ecotoxicological studies. Some β-adrenergic receptors have been characterized in fish using both traditional molecular cloning methods, or via mining of genomic sequences from various organisms. These approaches demonstrate that fish have β-adrenergic receptors very similar to those present in mammals. Since any effects of β-blockers in fish are most likely to be mediated via β-adrenergic receptors, it is the physiological processes regulated by these receptors that are most likely to be affected. Thus, cardiovascular dysfunction is one possible consequence of exposure of fish to these compounds, leading to impaired fitness (e.g., reduced growth and fecundity)[18]. In an experimental study on the spotted snakehead (*Channa punctatus*, Bloch) fishes, it was seen that when these fishes were exposed to high concentration (2 mM) of sodium arsenite (NaAsO), they died within 2.5 hr. The chromosomal DNA of liver cells were fragmented which suggest that NaAsO might induce death of those cells through apoptosis[19]. The toxicity of sodium cyanide (free cyanide) to the freshwater fish, *Labeo rohita* was studied and the LC_{50} of sodium cyanide in 96 hr was found to be 33 µg/L. The behavioural changes in fishes were also noticed after the lethal and sublethal doses of sodium cyanide[20].

Many experimental studies have been done to produce cancer in fish by different chemical carcinogens. Diethylnitrosamine (DEN), an N-nitroso (nitrosamine) compound, is one of the most potent carcinogens. Primary neoplasms with histological characteristics of HCC were observed in fish exposed to 125 ppm of DEN for 3-5 successive periods[21]. After administration of DEN (95 mg/L for 6 weeks), hemangiomas, cholangiomas, biliary cystadenomas and HCC were seen in the liver tissues of fish at the 18th week[22]. The rainbow trout (fish) were fed with a diet containing indole-3-carbinol (2000 ppm), β-naphthoflavone (500 ppm) or Aroclor 1254 (100 ppm) for 6 weeks before a single 24 hr exposure of an aqueous

solution of 250 ppm DEN. After 42 weeks, DEN produced 80.2% incidence of liver tumours. The tumour was inhibited by indole-3-carbinol but enhanced by β-naphthoflavone[23]. Liver cytotoxic alterations of adult medaka *(O. latipes)* fish were seen following short-term bath exposure (48 hr) to 500 mg/L DEN for 3-21 days[24]. Progression of hepatic neoplasia was observed in adult medaka fish (3-6 months old) following aqueous exposure to DEN (50 ppm for 5 weeks)[25]. The DEN-induced spongiosis hepatis (a hepatic lesion characterized by mutilocular cyst-like complexes) was noticed in the Japanese medaka, *O. latipes* (a small aquarium fish)[26]. The hepatomas were noticed in 6- and 9- month groups of the 100 ppm- and 200 ppm-DEN treated fishes. The guppies (*P. reticulata*) were exposed to multiple doses of DEN. The neoplastic foci of mixed hepatocytes and cholangiocytes increased in livers of guppies from the 2nd month, developing into hepatoblastomas, which occurred in almost 100% of guppies by the 12th month. The HCC was seen in rainbow trout fish exposed to the organochlorine insecticide, DDT. The neoplasms in rainbow trout exposed to N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) were seen in liver, stomach and kidney, with a low incidence in swim bladder. Methylazoxymethanol acetate (MAM-Ac), a potent carcinogen, also produced the cancer in fish, but to lesser degree than with MNNG. Liver was most commonly affected, followed by muscle (smooth and skeletal muscle, including heart), pancreas, connective tissues and kidney. The indirect-acting carcinogens such as polycyclic aromatic hydrocarbons (PAHs), with B[a]P and 7,12-dimethylbenzanthracene (DMBA) have also been evaluated to cause cancer in fish[1]. The rivulus fish was also used to induce the HCC by dietary antioxidant butylated hydroxyanisole[27].

The use of medicinal plants is an alternative to antibiotics in fish health management. The herbs are not only safe for consumers, but they also have a significant role in aquaculture. For this purpose, several experimental studies have been performed in different species of fish. The acute toxicity of Indian almond (*Terminalia catappa*) and garlic (*Allium sativum*) was tested in tilapia fingerlings. It was shown that these herbs have low acute toxicity and can treat the trichodiniasis caused by *Trichodina*. The immunostimulant effects of the dietary intake of *Viscum album, Urtica dioica and Zingiber officinale*) on rainbow trout (*Oncorhynchus mykiss*) have also been studied[28]. The immunostimulatory effect of aqueous extract of *Eclipta alba* (Bhangra) leaf in tilapia fish, *Oreochromis mossambicus* was observed[29]. The antibacterial activities of the methanolic extracts of 31 Brazilian plants against fish pathogenic bacteria have been screened out[30]. The immunostimulatory effects of 2 Chinese herbs (viz., *Lonicera japonica* and *Ganoderma lucidum*) were determined in tilapia fish (*Oreochromis niloticus*). On the basis of several studies, it was found that the oral administration of ginger (*Z. officinale*) extract increases the phagocytic capability of cells in rainbow trout, while the extracts of 4 Chinese herbs (*Rheum officinale, Andrographis paniculata, Isatis indigotica* and *Lonicera japonica*) increased the phagocytosis of white blood cells of carp[31]. The goldfish, *Carassius auratus* was used to observe the effects of *Phyllanthus niruri* and *Aloe vera* (Aloe), and it was found that these herbs can positively enhance the growth performance of fish, as well as they act against A. *hydrophila* infections. The synergistic effect of herbs has also been noted in other fish species, including Japanese flounder and *Clarias gariepinus*. The growth increase in *L. rohita* fish fed with herbal supplemented diet was also recorded. An experiment was also conducted on the *Catla catla* fish, and the disease resistant was produced through immersion treatment of 3 herbs, viz., *A. sativum*, *A. indica* and *Curcuma longa* (Haldi, turmeric) in spawn[32]. The Indian major carp, *L. rohita* fingerlings were experimented for the immunostimulatory and disease resistance effects of *Withania somnifera* (Ashwagandha) root against *A. hydrophila* infection[33]. The fingerlings of *L. rohita* fish were also used to evaluate the immunostimulant effects of the dietary intake of *A. sativum* and *Vitex negundo* extracts[34]. Several plant products seemed to be potent antiviral agents against fish viruses[35].

CONCLUSION

A number of experiments and the use of drugs have been performed in fish. The antibacterial, antiparasitic and anaesthetic drugs, besides the pharmacokinetic and pharmacodynamic parameters have been well experimented on the fish. Drugs, e.g., tetracyclines, penicillins, macrolides, quinolones, sulfonamides, immunostimulants, anticancer agents, herbal drugs, vaccines, etc. have been successfully experimented. Therefore, fish may be used as model organism in the experimental pharmacology and toxicology.

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