Fish models in experimental pharmacology: on the mark or off the mark?

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Fish has emerged as an alternative model organism in biomedical research for conducting experimental pharmacological and toxicological studies. As a vertebrate, it shares many conserved physiological and molecular features with humans making it a valuable model for diagnosing, investigating disease states and testing drugs to check toxicity and therapeutic activity against the target. Zebrafish and medaka are mainstream models that are widely employed in pharmaceutical research. This study aims to highlight the probability and potential of fish as an alternative model organism in biomedical research, drug discovery and development. Further, it discusses the limitations of fish models in experimental pharmacological and toxicological studies considering the changes in the residing environment, physiology, metabolism, unpredictable inter-individual variability due to diseases, variable conditioning, and interspecific and intraspecific variability.

Keywords: Drug screening, fish, model organism, pharmacology, toxicology.

Background

FISHES are the earliest, most abundant and diverse class of vertebrates, with approximately 34,000 species comprising 48% of the known member species of subphylum Vertebrata[1]. Although fishes diverged from humans approximately 400 million years ago, there are only a few differences at the molecular level, justifying the selection of fish as a model organism to conduct research relevant to humans[2]. The use of fish as a research model has been adopted worldwide to provide insights into complex human genetics, anatomical and physiological processes as well as the pathogenesis of human disorders[3].

For more than 200 years, fish has been used in experiments as a model organism, with gold-fish (*Carassius auratus*) being the oldest model species employed in toxicity studies. Thereafter, it because a popular model in fields such as growth, behavioural studies, immunology and reproduction[4]. Experiments conducted in the early 20th century promoted the emergence of medaka (*Oryzias latipes*) as a developmental genetic model organism. Medaka, like zebrafish (*Danio rerio*), has a completely sequenced genome, transparent embryos and adaptation to a wide range of temperatures and high fecundity, which enables it to be an extremely useful experimental animal in toxicology, developmental studies, disease modelling and environmental health sciences[5]. Zebrafish was first introduced as a biological model to study developmental genetics by George Streisinger in the 1960s. In recent years, it has successively emerged as the top research model with applicability extended to many other fields, including physiology, toxicology, disease modelling and drug development[6]. Presently, some other fish species are also in use as model organisms in experimental pharmacology, which includes large-sized species such as rainbow trout and small-sized ornamental fishes such as *Xiphophorus* sp., *Rivulus* sp., *Poecilia* sp. and family Cyprinodontidae.

The fish cell lines are also being developed as *in vitro* models to complement *in vivo* studies in related fields. About 283 cell lines have been established from finfish around the world[7]. Further, the increasing potential in using cells from lower vertebrates as *in vitro* models for studies of DNA repair function, makes fish a good model to monitor mutagenic and carcinogenic chemicals[8]. The potential for the application of research outcomes to both human as well as environmental health issues makes the fish species an attractive, demanding and valuable alternative model in carcinogenesis and toxicity studies[9].

Without scrutinizing the usefulness of current approaches which benefit from these fish models, especially zebrafish, the present strategies have some notable limitations to be taken care of. To begin with, there is a basic anatomical and physiological difference between humans and fish[10]. Although the fish is similar to humans genetically, there is a limit, as human diseases caused by genes that do not exist in fish or those affecting a specific tissue or body part that the fishes do not have will require another animal model[4,11]. Further, there can be interspecies variation as a response to drugs, which makes it difficult to extrapolate individual-level results under laboratory settings to the population level and then to humans[12]. Therefore, a detailed analysis is required first to assess the characteristics in order to compare the benefits and current limitations of using fish as a model organism in experimental pharmacology.

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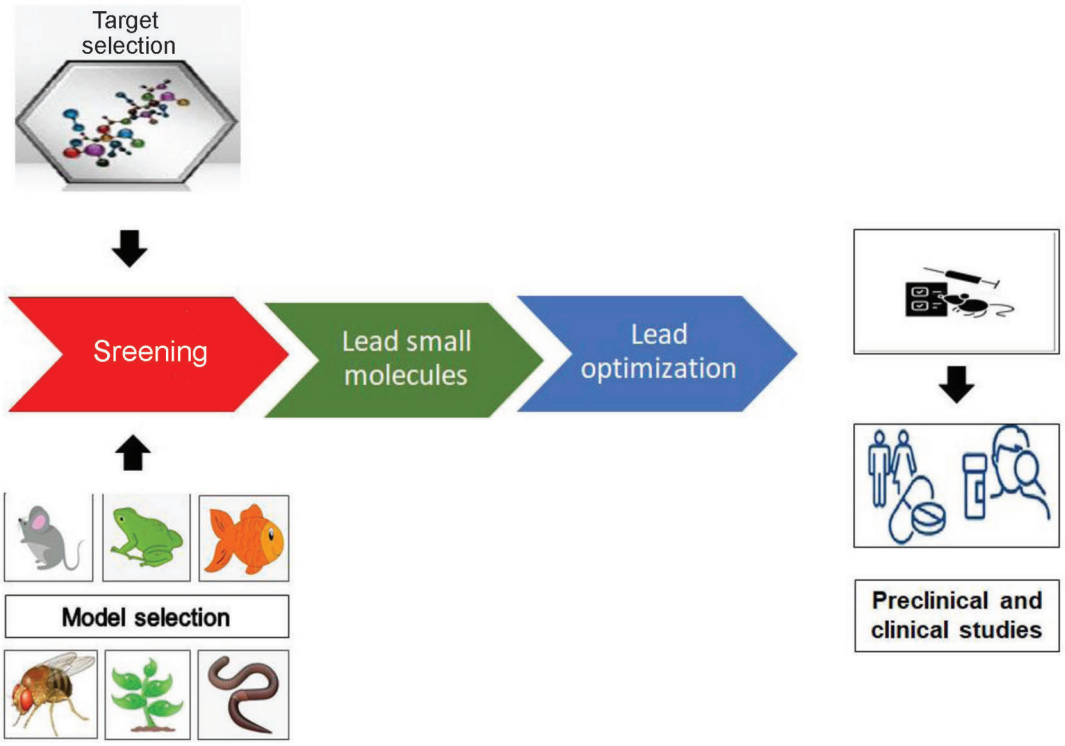

Figure 1. Drug screening strategies using target-based and phenotype-based approaches.

Thus, in this study, we analyse the scope of using the fish as a powerful model organism in experimental pharmacology along with its possible shortcomings. To strengthen this, we concentrate on gathering baseline information regarding the studies in which fish models are employed for understanding molecular and genetic mechanisms underlying human diseases, for determining drug efficacy and toxicity, drug screening and for assessing the adverse effects of known drugs. Further, this study gives an overview of the use of fish as a model organism in pharmaceutical research, drug discovery and development, and also provides insight to fish pharmacology and toxicology. Finally, we evaluate the limitations of fish models in experimental pharmacological and toxicological studies considering the changes in the residing environment, physiology, metabolism, unpredictable inter-individual variability due to disease, variable conditioning, and interspecific and intraspecific variability.

Applications of fish as a model species

Fish models can offer interesting possibilities for current and future biomedical research. This can serve as a gap-filler between *in vitro* assays and rodent testing.

As a platform for drug screening

Current drug discovery strategies utilize either an experimental phenotype-based or a rational target-based approach[13,14] (Figure 1). Both strategies have their own strengths and weaknesses. Nevertheless, phenotype screening only requires a little information on the disease or a validated target. It provides an insight into the overall efficacy of small molecules through simultaneous activity at multiple targets[15].

Model organisms such as zebrafish fill the gap between *in vitro* assays and expensive screenings using mammals. Since zebrafish is a small species and easy to maintain, the assays can be used in medium-to-high-throughput screening mode for pharmacological studies. Direct screening as well as screening of known drugs for new insights can be conducted using this model[16]. Screening technologies that exist as well as those being developed in zebrafish provide details of potential off-target effects of drug molecules on the cardiac system, central nervous system, intestinal tract, auditory and visual functions, pro-convulsing potential and osteogenesis. In zebrafish larvae, *in vivo* toxicology evaluation can be completed in 6–7 days. Hence the zebrafish model can be considered a useful pre-filter for identifying the safest lead candidates as early as possible in the drug discovery process[17].

Nowadays, the use of medicinal plants as a complementary treatment is gaining attention, whose validation requires experimental studies on animal models. For this, fish species-based models can be easily employed. The acute toxicity of Indian almond (*Terminalia catappa*) and garlic (*Allium sativum*) was evaluated in tilapia (*Oreochromis niloticus*) fingerlings and found to be less toxic. Therefore, these

herbs can be used as an alternative to treat the trichodiniasis caused by *Trichodina* sp.[18]. Similarly, rainbow trout (*Oncorhynchus mykiss*) fed with a diet containing the extract of *Viscum album*, *Urtica dioica* and *Zingiber officinale* showed non-specific immune responses[19]. *Eclipta alba* (Bhangra) leaf aqueous extract showed immunostimulatory effect in tilapia. Studies on Indian major carp (*Labeo rohita*) fingerlings using *Withania somnifera* also exhibited enhanced immunological and disease-resistance properties against *Aeromonas hydrophila* infection[7]. A study conducted on the neotropical freshwater fish, *Prochilodus lineatus* illustrated the acute lethal and sub-lethal effects of neem (*Azadiracta indica*) extract, which caused instability in the antioxidant defence system, and damaged fish gills and kidney tissues. These results prove that although neem extract is less toxic than synthetic pesticides, it causes functional and morphological changes in this species[20].

In regenerative medicine

Regenerative medicine is the branch of medicine that develops methods to heal or replace cells, tissues and organs damaged by age, disease or trauma, and normalize congenital disabilities. Currently, the impact of this field in clinical practice includes organ transplantation, skin grafting, generation and use of therapeutic stem cells and tissue engineering[21].

Fish can be a versatile model for studying regeneration as it can regrow many tissues and organs such as fins and heart. The first use of fish as a model organism was in the field of regenerative medicine for the regeneration of fins of goldfish[22]. Research in fish regeneration biology, focused largely on the zebrafish, has broadly expanded in recent years. Studying the mechanism of regeneration and homeostasis of tissues has given promising results relevant to the development of human regenerative medicine.

Zebrafish has a large regenerative capacity and is an ideal model for carrying out studies using cellular, molecular and genetic approaches. This model has the following advantages: it can restore organs that poorly regenerate in mammals, regeneration can be easily followed and multiple tools for genetic manipulations are possible. Table 1 summarizes some of the main injury models for studying regeneration in zebrafish[23–36].

Other fishes used as models for regeneration studies include *C. auratus* (goldfish), *Cyprinus carpio* (carp), *O. mykiss* (rainbow trout) and *Sternopygus macrurus* (yellow-stripe knifefish)[37].

Assessment of genotoxicity

Genotoxicity refers to the property of certain chemical substances that can produce deleterious effects on the genetic information within a cell. They can affect germ cells and pass on genetic changes to the next generation. Assessment of genotoxicity is an important component of drug development as well as toxicity studies. A genotoxic substance interacts directly or indirectly with the DNA causing strand breaks or additions or modifications[38]. Tilapia, *O. mossambicus* and zebrafish were used to estimate the DNA strand breaks induced by monocrotophos and DNA damage was recorded in treated samples (an organophosphate pesticide). Significant DNA damage was observed in all the treated fish compared to the control[39,40]. The genotoxic potential of Amikacin sulphate was recorded only at higher doses and longer exposures in *O. mossambicus*[41].

Synodontis clarias and *O. niloticus* were sampled from various locations/seasons of river Anambra and season, species of fish and geographical location affected the micronuclei profile of the fish[42]. A similar study was performed on *Astyanax altiparanae* to assess the geno-toxicity of samples from River Jordão with different levels of metal contamination. Micronucleus indices in fish erythrocytes after exposure to contaminated samples were measured and correlated with environmental parameters. An increase in water concentration of metals was observed in samples collected from the urban zone causing higher genotoxicity and hence greater impact[43]. Fish cell lines are an alternative to whole fish for assessment of genotoxicity in many toxicity studies. The cytotoxic effect of the organophosphorus pesticide, parathion was determined using FG-9307, a cell line derived from the gills of *Paralichthys olivaceus*. This study confirmed that the fish cell lines could be applied in acute *in vitro* cytotoxicity studies[44].

As a disease model

Fish as a biological model for diseases can be useful to elucidate biological mechanisms involved in the pathogenesis of disorders common to both fish and humans. Small-sized freshwater fish species that can be easily bred and maintained in large numbers at a lower cost are the most preferred. These species such as zebrafish, medaka, platy fish and swordtail fish are amenable to various molecular techniques

Table 1. Injury models for organ regeneration in fish

Organ	Type of injury	Reference
Caudal fin	Amputation	24
	Cryoinjury	25
Heart	Resection (ventricle – 20%)	26
	Genetic ablation	27
	Cryoinjury	28
Kidney	Chemical	29
Brain, spinal cord and eye	Spinal cord resection	30
	Stab lesion	31
	Optic nerve crush	32
Liver	Chemical	33
	Resection	34
Pancreas	Genetic ablation	35
Skin	Laser	36

that are valuable for modelling human disorders. Since at the molecular level there are very few differences, the application of a non-mainstream fish species as a disease model can also be explored with the aid of new and improved gene sequencing systems. Fish models can offer numerous experimental advantages, mainly ease of genetic manipulation, for studying disease mechanisms and other characteristics[45].

Zebrafish has been widely used as a candidate species for modelling human diseases. It shares notable genetic similarities with humans with approximately 70% of human disease genes having functional homologs in zebrafish. Myocardial infarction was modelled in zebrafish using cryoinjury. Regeneration of cardiomyocytes was studied in zebrafish heart and a protein, thymosin b4, was found to trigger the formation of new cardiac tissue. Zebrafish strains with mutations in the dystrophin gene were found to have a phenotype similar to the human disease, Duchenne muscular dystrophy (DMD). Since there is no cure for DMD, zebrafish DMD mutants have been used to screen potential compounds that can ameliorate the disease pathology[46]. A few studies report using zebrafish as a model for Parkinson's disease (PD) since it can mimic the phenomenology of different movement disorders. Zebrafish, being a highly social animal, lives in groups and therefore is particularly useful to model disorders of human social behaviour, such as aggression and autism. Continuous application of stress can develop zebrafish models of anxiety and depression due to its physiological and genetic homogeneity with human response to stress. Zebrafish is also used for assessing addiction, attention-deficit hyperactivity disorder and obsessive–compulsive disorder[47].

Medaka is complementary to zebrafish for modelling human diseases. The nonalcoholic steato-hepatitis model in medaka (*O. latipes*) was developed by feeding the fish a high-fat diet, exhibiting hyperlipidaemia, hyperglycaemia and degeneration of hepatocytes. The influence of *n*-3 polyunsaturated fatty acids on the disease progression was also studied[48]. Medaka can be a well-suited PD model as symptoms can be identified through behavioural analysis and verification of neuron loss. Both toxin- and mutation-induced PD models were developed in medaka fish[49].

The emerging fish models are evolutionary mutant models in which the disease developed is either adaptive or true illness. Antarctic fish, *Notothenia coriiceps* naturally exhibits reduced bone mineralization. Therefore, notothenioid genes responsible for natural osteopenia in this icefish are identified and characterized to better understand the mechanism involved in the pathogenesis of human bone diseases. The white-blooded icefish, *Chaenocephalus aceratus* is a species that has no erythrocytes or haemoglobin, but has special cardiovascular adaptations for an adequate supply of oxygen in the tissues. This can be a model for human anaemia and provides information regarding human red blood cell formation. Toadfish (*Opsanus tau*) provides models for hepatic encephalopathy and sickle cell anaemia by its characteristics of excessive urea production and similarity with mutant human sickle haemoglobin under hypoxia. Fish models which develop the same disease as in humans include platy and swordtail (melanoma), eels (bone demineralization, childhood kidney cancer) and damselfish (neurofibromatosis), etc.[2].

Nowadays, efforts to identify the disease pathogenesis as well as to reveal novel drug targets using model organism research have gained popularity. The application of dihydroorotate dehydrogenase inhibitors such as the anti-inflammatory drug leflunomide in melanoma formation was identified using the zebrafish model. Many more drug discovery studies using the zebrafish model have progressed to advanced stages of clinical studies. All-trans retinoic acid (ATRA) was found to suppress the transcription factor c-myb, a driver of adenoid cystic carcinoma using a pluripotent zebrafish blastomere culture system. The findings led to the phase-II clinical trial for testing the compound in patients with recurrent metastatic adenoid cystic carcinoma of the head and neck. ProHema is a compound currently being evaluated in an ongoing phase-II clinical trial in haematologic malignancies. Rosuvastatin, used for treating hypercholesterolaemia, has been repurposed as an antiangiogenic drug after a study using the zebrafish model. Furthermore, the morphological limb defects of thalidomide, as in humans, were detected using the zebrafish models[45].

Mycobacterium marinum causes a tuberculosis-like disease in fish, which can be employed as a cost-effective surrogate model for human tuberculosis (TB). Therefore, using medaka (*O. latipes*) to model chronic TB offers platforms for identifying anti-TB drugs[50].

Translation to clinical trials

Zebrafish has recently been used to develop 'avatar models' an emerging approach in precision medicine in oncology. In this, the cancer cells from a patient's tumour are xenotransplanted into zebrafish 'avatars' for drug efficacy studies and the results obtained can be translated to patients' trials. This ensures the best personalized drug-treatment for the patients. Recently, in an experiment conducted in zebrafish embryos, clotrimazole co-treatment with lonafarnib has been acknowledged as a potential cure for melanoma. Besides, xenotransplanted humanized models for respiratory diseases like SARS-CoV-2 infection can be developed in zebrafish owing to their swim bladders as buoyancy organs. Further, high-throughput screens using zebrafish models have helped discover new therapeutic candidates in many cases, where results of the clinical trial support the preclinical conclusion[51,52].

Currently used fish models in experimental pharmacology

Zebrafish is a widely accepted, versatile *in vivo* model organism for studying various topics ranging from developmental

biology and morphogenesis to neurosciences, regeneration and ageing. It has shown potential for drug discovery in various disease models. The zebrafish model was used to identify drugs limiting the cardiotoxicity of doxorubicin. A large number of compounds were screened, and visnagin and diphenyl urea were identified as cardioprotective compounds as they reduced the effects of doxorubicin on the zebrafish heart[13]. Medaka, another small, egg-laying, freshwater bony fish, can be considered a second laboratory fish model along with zebrafish. This is one of the small fish species of choice in many carcinogenesis bioassays[5]. Rainbow trout, a widely distributed freshwater fish species, is one of the widely studied fishes in many research areas, including carcinogenesis, toxicology, comparative immunology, disease ecology, physiology and nutrition. Rainbow trout as a large fish model in aflatoxin-induced hepatocellular carcinoma and medaka as the small fish species in diethyl nitrosamine-induced hepatocellular carcinoma show the potential of fish species as alternative models in carcinogenesis and toxicity research. Further, trout hepatocytes spheroids are identified to be promising *in vitro* models to study xenobiotic metabolism and drug efflux by assessing the expression and functionality of genes related to xenobiotic metabolism[9]. Many pharmacokinetic and pharmacodynamic studies have been done on different species of fish using several groups of drugs such as tetracyclines, penicillins, macrolides, quinolones, sulphonamides, immunostimulants, anticancer agents, herbal drugs and vaccines. Lamprey eels (predator family of jawless fish) have been employed as model organisms in spinal cord research. A novel protein was identified from the supraneural body of adult lamprey (LIP) that showed cytocidal activity against human MCF-7 and K562 cells with target cell specificity. LIP was found to have strong cytocidal effects against tumour cells both *in vitro* and *in vivo*. Silver nanoparticles are used for their antimicrobial properties, and their cytotoxic effect was assessed in the Indian carps, *Catla catla* heart cell line and gill cell line, and *Labio rohita* gill cell line using MTT (3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyl tetrazolium bromide) and neutral red assay. Goldfish was also successfully used for screening antibiotic drugs for neurotoxicity. Further, the molecular evolution of the opioid receptor family was studied using cDNAs isolated from the teleost fish, *Catostomus commersoni.* The study revealed that the function of opioid receptors in lower vertebrates is to suppress pain as in mammals, or they have developed an antinociceptive function during the course of evolution. Other fishes which have been used in biomedical research include: *Channa punctatus*, *Clarias gariepinus*, *Fundulus heteroclitus*, *Myoxocephalus scorpius*, *O. mossambicus*, *O. niloticus*, *Salmo trutta* and *Salmo iredius*[7]. Zebrafish is the most studied and preferred species to model humans, taking advantage of the orthologous genes of interest and the suitability for medium and high throughput screening. Sequencing of the zebrafish genome reveals that approximately 70% of human genes have at least one zebrafish orthologue, which makes it suitable for studying the genetics of organogenesis, embryo development as well as human physiology and disease[53]. Currently, the clinical side of biomedical research has introduced a trend known as personalized medicine that involves translating knowledge from preclinical models to humans. Co-clinical trial with zebrafish 'avatars' enables faster analysis of local and systemic effects of drug treatment for cancer and therefore, can be used for first-line therapy in spite of some technical challenges yet to be overcome[51].

It is essential to understand that there is no single fish model ideal for addressing all biomedical questions. Each species has unique strengths and weaknesses. Goldfish as a model system has advantages in understanding skeletal and organ morphology and colouration of vertebrates. Further, it is easy to collect molecular components from the blood and it is useful for micromanipulation experiments and establishing disease models[54]. In comparison with zebrafish, medaka is tough, less prone to diseases and has distinctly defined sex chromosomes[55]. Further, studies of medaka can provide information regarding additional phenotypes useful for disease modelling. Therefore, medaka can be a potential, complementary model organism in developmental biology and genetics[2]. Annual killifish has a short life span as an advantage for being a human ageing model[56]. Zebrafish is a successful and versatile model for studying developmental biology, diseases-like cancer, toxicology, drug discovery and molecular genetics[57]. Therefore, fish models have a promising role in advancing research in future (Figure 2).

Fish models in pharmacological and toxicological studies: are they feasible?

Model organisms are living, non-human species used during human medical investigations to gain knowledge about a disease, its prevention, diagnosis and treatment. The ease of experimenting under controlled situations and mimicking disease, conditions have reinforced the development of such models to be applied in various biological fields. With the need to restrict the use of animals in research and thereby minimize their suffering, lower organisms such as fish were proposed as an alternative model for study. Considering their short life span and high fecundity, they have entered the fray as model organisms for conducting experimental pharmacological and toxicological studies to understand the behaviour of the higher vertebrates[2]. It is well known that fishes are comparatively more affordable, easier to keep with a short-life cycle and faster to raise with high fecundity than mammals[1,3]. The eggs can be fertilized externally, easily manipulated and customized to the experimental requirements. Most of the egg stages are transparent, so that embryonic development can be easily monitored[58]. These physiological adaptations will facilitate the use of fish as a convenient model to diagnose and study disease states

and to test drugs for toxicity and their therapeutic activity against the target more effortlessly than in mammals[1,3]. Further, the close genetic similarities between fishes and humans help carry out genetic manipulation employing diverse methods and to study vertebrate development and human diseases[58]. Zebrafish is the emerging star among fish models, widely used in disease research and drug screening. Cytochrome p450 is a family of haemoproteins that codes for enzymes in drug metabolism. Zebrafish has 32 genes which are direct orthologs of those of humans. Notably, metabolic genes are better conserved than other genes between humans and fishes[59]. Resveratrol was found to improve age-related retinal neuropathy in zebrafish via activation of the AMPK, SIRT1 and mTOR signalling pathways that are involved in human ageing[60]. Large genetic screens of this species have applications in transgenesis, mutagenesis and early development studies.

However, the environment affects life over the course of evolution, from aquatic to terrestrial life, and such adaptation changes the structure and function of organ systems. Comparison of fishes and humans in terms of anatomy and physiological processes showed marked superficial differences as well as conserved genetic similarities[10]. Though mammals resemble humans better, fishes can be an alternative as they can offer to reduce the number of mammals sacrificed while supporting the results obtained through *in vitro* studies. Moreover, fishes offer flexibility in experiments; in case a particular species becomes unfit for experiments, there is always another fish species or one can shift to a mammalian model. For example, in developmental toxicity studies, fish models can only assess the direct effects of agents regarding reproduction and development, as the fish

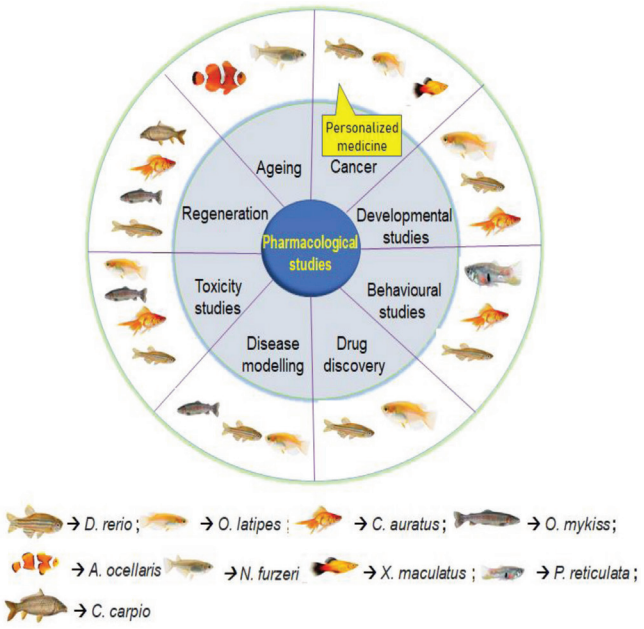

Figure 2. Applications of fish as a model organism in pharmacological studies.

does not include a placenta[61]. Further, regulatory challenges exist to using the fish as a model system. The Institutional Animal Care and Use Committee (IACUC) follows the guidelines for the care and use of laboratory animals, which ensures that the research animals are cared for and managed according to the highest possible standards. However, the guide needs to include more information relevant to fishes and other aquatic animals. In addition, the regulators, who are laboratory animal-care professionals, need to become more familiar with the fish as a research model. Hence, it is necessary to introduce fish-related issues to IACUC in order to familiarize with the need as well as a direct interaction between fish users and regulators in order to bridge the gap. Moreover, enhanced utilization of the presently available data, as well as compilation into a universal authoritative reference, can be recommended[62]. Presently, laboratory fish welfare needs more attention to preserve fish health and reduce inter-individual variability. Further, it is noticeable that even the species close evolutionary relationship with humans differs in important ways, which can affect the quality of using the fish as a stand-in model for humans. There is a basic difference in metabolism. Humans, being an endotherm, can keep their body temperature stable, whereas fish, an ectotherm, varies its body temperature depending on the environment. There can be numerous and unpredictable inter-species variability in terms of drug pharmacokinetics and pharmacodynamics[10,12]. Moreover, the influence of water temperature needs important consideration in heterotherms such as fish, as both pharmacokinetics and drug activities are temperature-dependent. Acclimatization is possible, provided the fish are kept at their respective acclimatization temperatures in the laboratory[12]. The laboratory fish welfare can be another challenge as the intensification of fish used in laboratory research, especially that of zebrafish raises ethical concerns[62]. It is also customary to monitor the water quality and other biological requirements when maintaining them in captivity, as these can influence their health and resulting research. Moreover, one cannot ignore their inter-individual variability due to disease, conditioning protocols, and interspecific and intraspecific variability[63].

Studies can become complicated due to skewed sex ratio in cohorts of zebrafish as sex determination in fish is often flexible, reversible and difficult to define by genetic factors. Moreover, the influence of environmental factors needs to be clearly understood[57]. For many genes in humans, there can be multiple additional forms in fish, such as two copies in zebrafish and four copies in goldfish. This complicates the generation of knock-out strains[64]. Moreover, only the zebrafish genome has been fully sequenced, for which a complete, comparative genetic analysis with that of humans is yet to be carried out.

Another drawback is the lack of placenta in ovoviviparous fish models, which results in direct interaction between chemicals or drugs and relevant tissues in contrast to indirect interaction through the placental connection in

humans. Last but not least, both differ from a physical standpoint, that is, fish possesses gills and human beings have lungs. Besides, fishes have two heart chambers while humans have four, which brings about the complexity of the circulatory system as fishes have a single circulatory system, whereas humans have double circulation. Therefore, care should be taken while modelling the complex congenital heart diseases in fish[65]. However, no perfect model exists other than the human being themselves. All model organisms have their benefits and drawbacks. Therefore, developing alternative model organisms can address the biomedical questions in a particular field of research.

Conclusion

No model organism can perfectly mimic human biology. However, fishes are widely used as an alternative model organism in preclinical studies, which could bridge the gap between *in vitro* analysis and the expensive *in vivo* screening using mammals. The model system is powerful yet not much explored in experimental pharmacology and provides an opportunity to have a better understanding of a disease, its therapeutic targets, drug toxicity and mechanisms of drug action. The practical use of fish models deals with different branches of science, such as regenerative medicine, genotoxic analysis and toxicity assessment. Fish models have been documented as potential candidates for imitating human diseases. From zebrafish and medaka, several fish species have been employed to identify different pharmacological targets. However, the current focus of fish models in pharmacology is mainly on nanomedicine, biosensors and genetic manipulation. Presently, zebrafish and medaka are the common small fish models in use. Genome editing technologies such as CRISPR and TALENs provide further possibilities for genetic manipulation. However, sequence annotation still needs to be completed and further research may enhance orthology. For fish models, replacing mammals completely as a preclinical model is limited in practical use due to the changes in the residing environment, physiology, metabolism, unpredictable inter-individual variability due to disease, variable conditioning, and interspecific and intraspecific variability. Currently, fish can offer a better alternative model for addressing biological questions from a comparative point of view. The exploitation of individual fish species being aware of the strengths and limitations of each should also be encouraged. Moreover, understanding the impact of environmental, behavioural, nutritional, genetic and epigenetic factors on research outcomes can take time and effort. Further, it is anticipated that future studies may reveal more possibilities for manipulating this model for pharmacological applications.

Conflict of interest: The authors declare that they have no conflict of interest.

1. Bolis, C. L., Piccolella, M., Dalla Valle, A. Z. and Rankin, J. C., Fish as model in pharmacological and biological research. *Pharmacol. Res*., 2001, **44**, 265–280.
2. Schartl, M., Beyond the zebrafish: diverse fish species for modeling human disease. *Dis. Models Mech.*, 2014, **7**, 181–192.
3. Schaeck, M., Broeck, W. V. D., Hermans, K. and Decostere, A., Fish as research tools: alternatives to *in vivo* experiments. *Altern. Lab. Anim*., 2013, **41**, 219–229.
4. Teame, T. *et al*., The use of zebrafish (*Danio rerio*) as biomedical models. *Anim. Front*., 2019, **9**, 68–77.
5. Hilgers, L. and Schwarzer, J., The natural history of model organisms: the untapped potential of medaka and its wild relatives. *eLife*, 2019, **8**, e46994.
6. Simonetti, R. B., Marques, L. S., Streit, D. P. and Oberst, E. R., Zebrafish (*Danio rerio*): the future of animal model in biomedical research. *J. FishSci.com*, 2015, **9**, 39–45.
7. Pandey, G., A review of fish model in experimental pharmacology. *Int. Res. J. Pharm*., 2011, **2**, 33–36.
8. Law, J. M., Mechanistic considerations in small fish carcinogenicity testing. *Inst. Lab. Anim. Res. J*., 2001, **42**, 274–284.
9. Bunton, T. E., Experimental chemical carcinogenesis in fish. *Toxicol. Pathol*., 1996, **24**, 603–618.
10. van de Pol, I., Flik, G. and Gorrisen, M., Comparative physiology of energy metabolism: fishing for endocrine signals in the early vertebrate pool. *Front. Endocrinol*., 2017, **8**, 36.
11. Lin, C. Y., Chiang, C. Y. and Tsai, H. J., Zebrafish and medaka: new model organisms for modern biomedical research. *J. Biomed. Sci*., 2016, **23**, 19.
12. Toutain, P. L., Ferran, A. and Melou, A. B., Species differences in pharmacokinetics and pharmacodynamics. *Handb. Exp. Pharmacol*., 2010, **199**, 19–48.
13. Strange, K., Drug discovery in fish, flies, and worms. *Inst. Lab. Anim. Res. J*., 2016, **57**, 133–143.
14. Szabo, M. *et al*., Cell and small animal models for phenotypic drug discovery. *Drug Des. Dev. Ther*., 2017, **11**, 1957–1967.
15. MacRae, C. A. and Peterson, R. T., Zebrafish as tools for drug discovery. *Nature Rev*., 2015, **14**, 721–731.
16. Giacomotto, J. and Ségalat, L., High-throughput screening and small animal models, where are we? *Br. J. Pharmacol*., 2010, **160**, 204–216.
17. Caballero, M. V. and Candiracci, M., Zebrafish as screening model for detecting toxicity and drug's efficacy. *J. Unexplor. Med. Data*, 2018, **3**, 4.
18. Chitmanat, C., Tongdonmuan, K. and Nunsong, W., The use of crude extracts from traditional medicinal plants to eliminate *Trichodina* sp. in tilapia (*Oreochromis niloticus*) fingerlings. *Songklanakarin J. Sci. Technol*., 2005, **27**, 359–364.
19. Dugenci, S. K., Arda, N. and Candan, A., Some medicinal plants as immunostimulant for fish. *J. Ethnopharmacol*., 2003, **88**, 99–106.
20. Winkaler, E. U., Santos, T. R. M., Machado-Neto, J. G. and Martinez, C. B. R., Acute lethal and sublethal effects of neem leaf extract on the neotropical freshwater fish *Prochilodus lineatus*. *Comp. Biochem. Physiol. Part C*, 2007, **145**, 236–244.
21. Atala, A., Regenerative medicine strategies. *J. Pediatr. Surg*., 2012, **47**, 17–28.
22. Gemberling, M., Bailey, T. J., Hyde, D. R. and Poss, K. D., The zebrafish as a model for complex tissue regeneration. *Trends Genet*., 2013, **29**, 1–19.
23. Marques, I. J., Lupi, E. and Mercader, N., Model systems for regeneration: zebrafish. *Development*, 2019, **146**, 1–13.
24. Pfefferli, C. and Jazwinska, A., The art of fin regeneration in zebrafish. *Regeneration*, 2015, **2**, 72–83.
25. Chassot, B., Pury, D. and Jaźwińska, A., Zebrafish fin regeneration after cryoinjury-induced tissue damage. *Biol. Open*, 2016, **5**, 819–828.

26. Yu, F., Li, R., Parks, E., Takabe, W. and Hsiai, T. K., Electrocardiogram signals to assess zebrafish heart regeneration: implication of long QT intervals. *Annu. Rev. Biomed. Eng*., 2010, **38**, 2346–2357.
27. Wang, J. *et al*., The regenerative capacity of zebrafish reverses cardiac failure caused by genetic cardiomyocyte depletion. *Development*, 2011, **138**, 3421–3430.
28. Chablais, F., Veit, J., Rainer, G. and Jaźwińska, A., The zebrafish heart regenerates after cryoinjury-induced myocardial infarction. *BMC Dev. Biol*., 2011, **11**, 21.
29. Reimschuessel, R. A., Fish model of renal regeneration and development. *ILAR*, 2001, **42**, 285–291.
30. Goldshmit, Y., Sztal, T. E., Jusuf, P. R., Hall, T. E., Nguyen-Chi, M. and Currie, P. D., Fgf-dependent glial cell bridges facilitate spinal cord regeneration in zebrafish. *Neuroscience*, 2012, **32**, 7477–7492.
31. Marz, M. *et al*., Regenerative response following stab injury in the adult zebrafish telencephalon. *Dev. Dyn*., 2011, **240**, 2221–2231.
32. Diekmann, H., Kalbhen, P. and Fischer, D., Characterization of optic nerve regeneration using transgenic zebrafish. *Front. Cell. Neurosci*., 2015, **9**, 118.
33. North, T. E. *et al*., PGE2-regulated Wnt signaling and *N*-acetylcysteine are synergistically hepatoprotective in zebrafish acetaminophen injury. *Proc. Natl. Acad. Sci. USA*, 2010, **107**, 17315–17320.
34. Kan, N. G., Junghans, D., Izpisua and Belmonte, J. C., Compensatory growth mechanisms regulated by BMP and FGF signaling mediate liver regeneration in zebrafish after partial hepatectomy. *FASEB J*., 2009, **23**, 3516–3525.
35. Ye, L., Robertson, M. A., Mastracci, T. L. and Anderson, R. M., An insulin signaling feedback loop regulates pancreas progenitor cell differentiation during islet development and regeneration. *Dev. Biol*., 2016, **409**, 354–369.
36. Richardson, R. *et al*., Adult zebrafish as a model system for cutaneous wound-healing research. *J. Invest. Dermatol*., 2013, **133**, 1665.
37. Unguez, G. A., Electric fish: new insights into conserved processes of adult tissue regeneration. *J. Exp. Biol*., 2013, **216**, 2478–2486.
38. Bhat, S. A., Scenario of genotoxicity in fishes and its impact on fish industry. *IOSR-JESTFT*, 2014, **8**, 65–76.
39. Banu, B. S., Danadevi, K., Rahman, M. F., Ahuja, Y. R. and Kaiser, J., Genotoxic effect of monocrotophos to sentinel species using comet assay. *Food Chem. Toxicol*., 2001, **39**, 361–366.
40. D'Costa, A. H., Shyama, S. K., Kumar, M. K. P. and Fernandes, T. M., Induction of DNA damage in the peripheral blood of zebrafish (*Danio rerio*) by an agricultural organophosphate pesticide, monocrotophos. *Int. Aquat. Res*., 2018, **10**, 243–251.
41. Dash, M. R. and Soren, D., Testing of genotoxic potential of amikacin sulphate through micronucleus test in a fish *in vivo* system. *Int. Res. J. Biol. Sci*., 2018, **7**, 36–40.
42. Obiakor, M. O., Okonkwo, J. C. and Ezeonyejiaku, C. D., Genotoxicity of freshwater ecosystem shows DNA damage in preponderant fish as validated by *in vivo* micronucleus induction in gill and kidney erythrocytes. *Mutat. Res.-Genet. Toxicol. Environ. Mutagen*., 2014, **775–776**, 20–30.
43. Francisco, C. D. M., Bertolino, S. M., De Oliveira, R. J., Morelli, S. and Pereira, B. B., Genotoxicity assessment of polluted urban streams using a native fish *Astyanax altiparanae*. *J. Toxicol. Environ. Health*, **82**, 514–523.
44. Li, H. and Zhang, S., *In vitro* cytotoxicity of the organophosphorus pesticide parathion to FG-9307 cells. *Toxicol. Vitro*, 2001, **15**, 643–647.
45. Patton, E. E., Zon, L. I. and Langenau, D. M., Zebrafish disease models in drug discovery: from preclinical modelling to clinical trials. *Nature Rev. Drug Discov*., 2021, **20**, 611–628.
46. Santoriello, C. and Zon, L. I., Hooked! Modeling human disease in zebrafish. *J. Clin. Invest*., 2012, **122**, 2337–2343.
47. Meshalkina, D. A., Kysil, E. V., Warnick, J. E., Demin, K. A. and Kaluef, A. V., Adult zebrafish in CNS disease modeling: a tank that's half-full, not half-empty, and still filling. *Lab. Anim*., 2017, **46**, 378–387.
48. Matsumoto, T., Terai, S. and Oishi, T., Medaka as a model for human nonalcoholic steatohepatitis. *Dis. Models Mech*., 2010, **3**, 431–440.
49. Matsui, H., Gavinio, R. and Takahashi, R., Medaka fish Parkinson's disease model. *Exp. Neurobiol.*, 2012, **21**, 94–100.
50. Walter, R. B. and Obara, T., Workshop report: the medaka model for comparative assessment of human disease mechanisms. *Comp. Biochem. Physiol. Part C*, 2015, **178**, 156–162.
51. Usai, A. *et al*., A model of a zebrafish avatar for co-clinical trials. *Cancers (Basel)*, 2020, **12**, 677.
52. Precazzini, F. *et al*., Automated *in vivo* screen in zebrafish identifies clotrimazole as targeting a metabolic vulnerability in a melanoma model. *Dev. Biol*., 2020, **457**, 215–225.
53. Stewart, A. M. *et al*., Molecular psychiatry of zebrafish. *Mol. Psychiatry*, 2015, **20**, 2–17.
54. Ota, K. G. and Abe, G., Goldfish morphology as a model for evolutionary developmental biology. *WIREs Dev. Biol.,* 2016, **5**, 272–295.
55. Kondo, M., Nanda, I., Schmid, M. and Schartl, M., Sex determination and sex chromosome evolution: insights from medaka. *Sex. Dev*., 2009, **3**, 88–98.
56. Herrera, M. and Jagadeeswaran, P., Annual fish as a genetic model for aging. *Biol. Sci*., 2004, **59**, 101–107.
57. Hoo, J. Y., Kumari, Y., Shaikh, M. F., Hue, S. M. and Goh, B. H., Zebrafish: a versatile animal model for fertility research. *BioMed. Res. Int.*, 2016, **2016**, 9732780.
58. Bradford, Y. M. *et al*., Zebrafish models of human disease: gaining insight into human disease at ZFIN. *Inst. Lab. Anim. Res. J*., 2017, **58**, 4–16.
59. Li, S. *et al*., Constructing a fish metabolic network model. *Genome Biol*., 2010, **11**, 115.
60. Wang, N. *et al*., Exploration of age-related mitochondrial dysfunction and the anti-aging effects of resveratrol in zebrafish retina. *Aging*, 2019, **11**, 3117–3137.
61. Dugershaw, B. B., Aengenheister, L., Hansen, S. S. K., Hougaard, K. S. and Thurnherr, T. B., Recent insights on indirect mechanisms in developmental toxicity of nanomaterials. *Part. Fibre Toxicol*., 2020, **17**, 31.
62. Lawrence, C. *et al*., Regulatory compliance and the zebrafish. *Zebrafish*, 2009, **6**, 453–456.
63. DeTolla, L. J. *et al*., Guidelines for the care and use of fish in research. *Inst. Lab. Anim. Res. J*., 1995, **37**, 159–173.
64. Malaga-trillo, E., Laessing, U., Lang, D. M. and Stuermer, C. A. O., Evolution of duplicated reggie genes in zebrafish and goldfish. *J. Mol. Evol*., 2002, **54**, 235–245.
65. Lee, H. C., Lin, C. Y. and Tsai, H. J., Zebrafish, an *in vivo* platform to screen drugs and proteins for biomedical use. *Pharmaceuticals*, 2021, **14**, 500.

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