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Zebra Fish Model as an Alternative to Animal Testing: A Review

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ABSTRACT

Zebrafish (*Danio rerio*) is a freshwater fish that belongs to the family of small fish (*Cyprinidae*) of the order Cypriniformes. Originally from South Asia, it is a popular aquarium fish, which is often sold under the trade name zebra danio. Zebrafish is an important vertebrate model organism and is generally used in scientific research, such as, in drug development, especially in preclinical development. This is also important for its regenerative capacity, and has been modified by researchers to produce many transgenic strains. In this review, we have compiled the information of how zebra fish model useful as an alternative to animal testing for various diseases, such as cancer, cardiovascular diseases, diabetes, obesity, neurological and kidney disorders etc.

Keywords: *Zebra fish; Danio rerio; Preclinical trial; Clinical trial*

INTRODUCTION

In Pharmacy drug discovery is continuously process in which various scientist try to discover continuously a new scaffold which is more prominent pharmacological action as compare to exciting drug with a minimum side effect. After finding a new scaffold drug are need to pass various stages like preclinical trial. During preclinical trial we try new scaffold on various types of animal model such as mice, frog, and Guinea pig. From 1960 scientist is used a zebra fish model for clinical trial. In all world large number of animals are scarifies due to the new drug unknown toxicities. Overall scientist is trying to prepare a safer drug with minimum toxicity but it still it produces large number of sacrifices. Due to this it makes more unpredictable and costly process [1]. So, by intending streamlining toxicological study by applying safety majors many scientists choose different path by consider alternative animal model such as zebra fish model. From last 40 years, it is appeared promising model for reviewing verity of human diseases because of its genome sequence are resembles with human genome sequence with near about 87% similarities and considered as 70% of human genes are orthologues gene in zebra fish model [2,3].

The zebra fish is also called as *Danio rerio* which found in fresh or clean water (Figure 1). Zebra fish belonging to family *Cyprinidae*. Commonly found in south asian countries as a tropical fish. The zebra fish is about 2.5 cm - 4 cm in length. In early stages it is transparent in nature but when it matures it develop characteristic strips all over a body. Look attractive with bluish red coloration. The male zebra fish smear and torpedo like shape having pink trace. Where as in female zebra

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fish has fewer pink traces with blue strips and heavier than male zebra fish. Zebra fish has 2-3 year life span but in ideal condition it is extended to 5 years [4,5].

Figure 1: Zebra fish.

Many genes from human diseases are associated with zebra fish with mutation similarities zebra fish model is convenient model for human diseases such as cancer, cardio-vascular diseases, Neurological disorder. Zebra fish have large similarities in many blood cell type such as red blood cells, neutrophils, Macrophages, monocytes, T and B cells also have equivalent of platelet it is possible to use zebra fish model in hematological disorder. In an interesting note, investigating gene function and modeling a verity of human diseases, zebra fish embryos has been extensively used in cardio-toxicity study. Zebra fish embryos seems to be useful in toxicological studies [6,7].

As compare to other animal model this model is inexpensive with high output experimental model with several benefit such as quantity of tested agent is reduced, duration of experiments is also reduced with promising results. Many pharmaceutical dosages forms are withdrawn from market due to drug induce cardiotoxicities and shows minimum drug safety. Which make costly process? To minimize the cost there is a need to identify the potential adverse drug response (ADR) before reaching to clinical trials. Clinical researcher and academician's now showing interest in zebra fish model. It is creating a link culture assay (Inexpensive with less data) and other mammalian model (expensive with large data) in pre-clinical study. Toxicity study reported by zebra fish model give close insight to the human biology than any other in vitro model [8,9].

Danio rerio model also shows promising model in the cancer because of its smaller size, heavy offspring with rapid mutation rate as compared to murine model. With extensive range assay is carried out by target discovery, target validation and toxicological studies. It generates large number of offspring's it leads to generate confidence in statistical analysis. It also share high grade similarities human proteins that are responsible for cancer disease give unique advantage. It also reduces husbandry expenses as compare to the other mammalian models. This model gives viability in drug administration because zebra fish absorb water soluble drug or molecules [10].

Zebra fish provide 3Rs in drug discovery

Alternatives to animal testing were planned to overcome some of the drawbacks related with animal experiments and evade the unethical procedures. A policy of 3 Rs is being applied which stands for reduction, refinement and replacement of laboratory use of animals [11,12].

The 3Rs: replacement, reduction, and refinement of animal studies in research have been developed, over time, along with philosophies underlying the use of animals, regulatory directives, and novel technologies, the latter of which can be helpful

to science and animal welfare. Among novel approaches, the zebra fish have arisen as a popular alternative animal model [13,14].

Replacement: Zebra fish experiments with zebra fish larvae can be used to replace several animal toxicity studies, first establishing that zebra fish larvae are relevant models for systems with validation studies.

Reduction: As a first-level toxicity model, zebra fish larvae can be used to identify candidates for toxic drugs, allowing safer molecules to be analyzed in mammalian models. Ultimately, this will reduce the number of animals used in testing.

Refinement: Zebra models of embryos and larvae offer improvements to animal research designs, because embryos are fertilized externally and transparently for the first few days of life. This allows the observation of non-invasive toxicity and possibly recovery [38]. The various zebra fish models are shown in Figure 2.

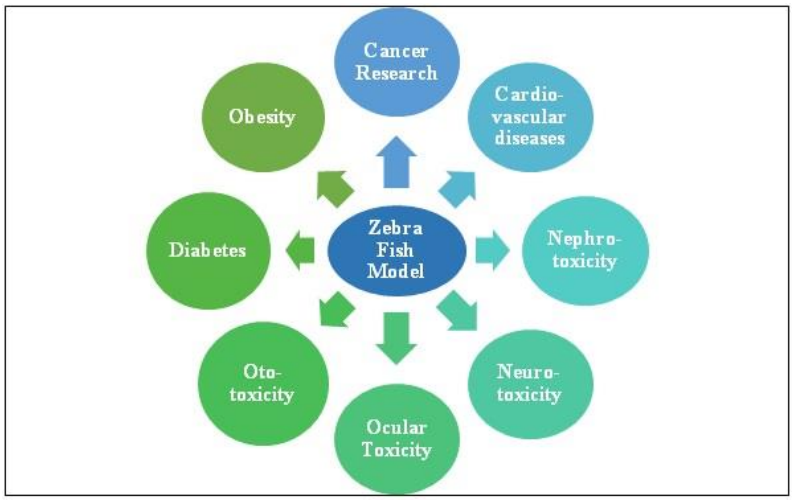

Figure 2: Zebra fish models.

Zebra fish model in cancer research

Zebra fish was first used in cancer research during the 1960s to test the effects of carcinogens [15]. Zebra fish model has various advantages in study of variety of different type of cancer therapy over other cell culture assay method. In zebra fish and human have share common pathways of tumor development because they have similar offspring mutation rate. It spawns large number of embryos which helps in providing large number of data for statistical data. Over 130 distinct gene have similar appearance or similar mutation rate with liver tumor cell with various similarities such as histology and progression stages [8,16,17].

Zheng et al. [18] suggest that zebra fish model has share similar molecular sign as human liver carcinoma. There are various different methods to generate human carcinoma in zebra fish like transplantation of tumor cell, development of mutant etc. which give exact insight about development of tumor because of these mutation rate. It also gives clear view about migration tumor form one part to another and metastasis of cancer cell.

Zebra fish model also have advantage over murine model due to its maintenance cost is very less, with side range of work feasibility because of fast embryo formation, and easy to obtain cancer phenotype. Murine model Require four months to

tumor formation where as in zebra fish model time limited to only 15-30 days. It provides transparency of the embryo and progress in of mutants without pigmentation gives possibilities to visualize all the cancer process [19-21].

In zebra fish model tumor develops is almost same as human tumor with similar morphology with same gene expression. It greatly facilitates to observe the response drug in zebra fish model over the model; it proves effectiveness of this model. Whereas tumor spectra found different when you compared to mice and human. Due to this promising characteristic it plays important role in cancer therapy [22].

Zebra fish model in cardio-vascular diseases

In a last ten years, the zebra fish use as popular method for various cardiovascular diseases. It has several advantages over other animal model such as zebra fish model has comparatively low-cost model as compared to other animal model. The embryo in zebra fish model is transparent which is sufficient enough to give good insight of model. Embryo in zebra fish model develops externally with large number with high richness useful in large number of genetic screening. The zebra fish shows similar response to various kinds of medicinal agent as shown in various another mammalian models [22-25].

In zebra fish model heart is located in front and towards lower surface of thoracic cavity between the operculum and the pectoral girdle. The heart of zebra fish cover with silver color sac called as pericardium. Within the pericardium it contains four chamber of heart such as atrium, ventricles, sinus venous and out flow track such as *Bulbus arteriosus*. Heart morphology, other development stages of heart and gene progression of heart are similar to the mammalian model. Now a days the embryo of zebra fish use for various cardiotoxicity studies. Such as quinidine and thioridazine shows QT prolongation effect on human body where as it shows AV block effect on zebra fish model. Likewise, metoprolol and phenytoin show decrease heart rate effect on human as well as on zebra fish [26,27].

Zhu et al. [26] suggest that zebra fish shows pericardial edema and circulatory disturbance in response to various cardio-toxic drugs. He tested seven well known cardiotoxic drug by zebra fish model such as aspirin, clomipramine hydrochloride, cyclophosphamide, nimodipine, quinidine, terfenadine and verapamil hydrochloride. As we know aspirin are used to raise heart rate. And other drug is responsible for slower heart rate. Drug administered to zebra fish model by yolk sac microinjection. After four and twenty four hours drug response recoded by the parameter of heart rate, heart rhythm, pericardial edema, circulation hemorrhage and thrombosis six parameter. After evolution it found that effect of drug on human and zebra fish are equivalent. Hence, he concludes that zebra fish model is an outstanding model for cardiotoxicity study.

A Albini et al. [28] used a zebra fish model for screening for drug induces cardiotoxicity. He uses several drugs such as 5-flurouracile, mitoxantrone, doxorubicin and cyclophosphamide from anti-cancer category and terfenadine from anti-histamine category. Drug concentration ranges from 0.01µM-100µM administer to zebra fish. And after twenty-four-hour cardiotoxicity assessed on criterion of heart rate, heart rhythm, circulation found similar effect. Also, it found that antibiotics such as gentamicin and anti-viral agent such as amantadine also shows similar effect in human & zebra fish model.

Over all finding suggest that zebra fish model plays important role in preclinical trial, also it shows significant model in testing cardio toxicity, and become common model system for cardiovascular diseases.

Zebra fish model in neurotoxicity

Zebra fish model now days widely used to study drug behavior and its pharmacological action of neurological disorder. The arrangement/ function of central nervous system and major subdivision of brain is similarly ordered as other vertebrates. It is also found that nervous system zebra fish has close association with human nervous system like neurotransmitter system such as GABA, dopamine, epinephrine, nor epinephrine, serotonin and histamine are also present in zebra fish [29,31]. And these are act as a prime target for pharmacological and toxicological activities. For epileptic study it needs to close view on locomotion activities that relate to brain function and nervous system. Automatic tracking study gain high attention. Adult zebra fish able to show the response such as social interaction and learning attitude [32].

Zebra fish model gives significant result as compare to other animal model for example pentylenetetrazol and picrotoxins causes dose dependent locomotors activity increases where as anti-epileptic drug such as diphenylhydantoin and valproic acid produce decrease locomotors activities. This effect of drugs on zebra fish model is comparable to the response observed on rodent model [33].

Adult zebra fish model is used to identify the anxiety effect of novel molecules in clinical trials; in this they used common test such as novel tank test (NTT) and Light dark test (LDT). This test is similar to the test carried on rodent model. Where environmental factor plays as important role to carry out this activity on zebra fish model there is need to carry in a dark place or environmental condition [34].

Over all study suggest that, zebra fish model is the promising model for neurological study. It uses same biological target which resembles to human body. In vitro model embryo allows the scientist to observe conserved protein by fluorescent tags; it also uses to study genetic manipulation.

Zebra fish model in nephrotoxicity

The kidney is prime organ to remove toxic materials form body. Toxic material removes from circulation by globular filtration. Toxic material severely affects the functioning of kidney. And result in to kidney failure. To study the effect on various nephrotoxic materials and kidney function and histology zebra fish model is a coinvent model [35].As compared to kidney functioning and anatomy in zebra fish are resembles with human as compared to the other animal model. Due to these facts zebra fish (*Danio rerio*) is good option to other mammalian model which gives advantage to apply powerful genetic experiment methods by using this model [36].

In the case of kidney this model proves to be applicable and versatile model due to the histological implicitly of pronephros, which contains two nephrons that possess conserved structural and physiological aspect with mammalian nephrons [37]. Many scientists suggest that larval zebra fish is shows promising effect in drug induce nephrotoxicity. Fluorescent tracer method (FITC) and electron microscopy methods are used for determination of drug induce nephrotoxicity [35,36].

The three drug such as paracetamol (NSAID), gentamicin (6-fluro quinoline class anti-bacterial agent) and tenofovir (anti-viral agent) are responsible for inflammation on renal tubules of the nephron that effect also shown in zebra fish model by change in morphological and histological changes in kidney of zebra fish larvae. Likewise, the kidney injury with a drug such as glutathione and purine are also found in zebra fish model [38,39].

Myoung JK [40] used this model to study cisplatin nephrotoxicity. As we know cisplatin is used to treat various type of cancer but it produces major side effect, nephrotoxic acute kidney injury. When he developed model to induce cisplatin nephrotoxicity in zebra fish, he found that intraperitoneal injection of cisplatin caused a decline in kidney proximal tubular functions. After study he conclude that zebra fish model is adult zebra fish are not only suitable for drug screening and genetic manipulation but as a simplified but powerful model to study pathophysiology of cisplatin nephrotoxicity.

Zebra fish model in ocular toxicity

Zebra fish has anatomical similarities with human eye such as cornea, lens, choroid and retina with similarities including genetic expression and tissue structure. Zebra fish has color vision owing to cone dense area which is similar to the humans. Such as similarities leads a zebra fish model is promising pharmacological model for preclinical trials [41,42].The optokinetic assay, dark and light alternating vertical strips are the two assay used for determining ocular toxicity by using adult zebra fish model. The few drugs such as chlorpromazine, cisplatin, gentamicin, minoxidil and vardenafil show no ocular toxicities as similar to the human with high sensitivity specifically to 75%-100% [38].

Zebra fish model in ototoxicity

Drug-induced ototoxicity is often either reversible, or sometimes not, which leads to a serious impact on the quality of life of patients without alternative medicine, such as aminoglycosides for life-threatening infections and cisplatin for cancer therapy. Detection of ototoxicity in preclinical mammalian models is very difficult and with some regulatory requirements, this is rarely done. The zebra fish model for ototoxicity can fill these gaps in preclinical safety testing. Zebra fish model is useful due to its homologous hair cells physiology, ear anatomy and neuronal signaling are similar with human systems as well as supported conserved genetic regulation [38,43]. The superficial location of the hair cells in the zebra fish allows controlling the changes in the hair cells in vivo by means of high-throughput method. This makes the zebra fish an extremely popular model for ototoxicity. Numerous drugs such as aminoglycoside antibiotics, carbonic anhydrase inhibitors, platinum-based chemotherapy, and environmental metal contaminants are ototoxic to humans are ototoxic for developing, larval and adult zebra fish [44-46].Therefore the zebra fish model is a promising tool to find out new chemical entities for ototoxicity.

Zebra fish model in diabetes

The development of the zebra fish pancreas is highly homologous to mammals, such as mice. The signaling mechanism as well as the way the pancreas works is very similar. The pancreas has endocrine compartments, which contain several cells. Insulin-producing β cells and Pancreatic PP cells that produce polypeptides are two examples of these cells. The structure of the pancreas, along with the glucose homeostasis system, is extremely useful for studying diseases, such as diabetes, that are related to the pancreas. Models for pancreatic function, such as fluorescent protein staining are useful in determining the process of glucose homeostasis and pancreatic development. Glucose tolerance tests have been developed using zebra fish and now it can be used to assess glucose intolerance or diabetes in humans. Insulin function is also being tested in zebra fish, which in turn will contribute to human medicine. Most of the work done on glucose homeostasis knowledge comes from work on the zebra fish that is transferred to humans [47-49].

Zebra fish model in obesity

Zebra fish have been used as a model system to study obesity, with research on genetic obesity and obesity caused by excess nutrition. Fat zebra fish, similar to fat mammals, show deregulation of lipid control metabolic pathways, which leads to weight gain without normal lipid metabolism. As like mammals, zebra fish stores excess fat in visceral, intramuscular, and subcutaneous area of the body. Due to such reasons zebra fish a good model for studying obesity in humans and other species. Genetic obesity is commonly studied in transgenic zebra fish or mutated with obesogenic genes. For example, transgenic zebra fish with over expressed AgRP, an endogenous melanocortin antagonist, exhibiting increased body weight and adipose deposition during growth. Although zebra fish genes may not be exactly the same as human genes, this test can provide important information about possible genetic causes and treatments for human genetic obesity. The zebra fish model with obesity caused by diet is very helpful because the diet can be changed from a very young age. High-fat diets and general excessive diets show a rapid increase in fat accumulation, increased BMI, hepatosteatosis, and hypertriglyceridemia. However, samples with normal excess fat remain metabolically healthy, whereas dietary samples with high fat content do not. Understanding the differences between types of obesity induced by diets can be beneficial in human care for obesity and related health conditions [47,50].

CONCLUSION

Due to the promising result in preclinical trial scientist increase the use of zebra fish model in their preclinical trial in 2018. Among 3000 scientific publication contains zebra fish model which is continuously increases and from last 10 years it increases by 3 times. Zebra fish model is used to replace few animal toxicities study which useful and promising model than another animal model. It is used to find out toxicity of drugs which reduce quantity of animals used for toxicology of drug. It is also suited for identification of novel targets associated with the risk and treatment of cancer, cardiovascular diseases, diabetes, obesity, neurological and kidney disorders etc.

CONFLICTS OF INTEREST

Author declares no conflicts of interest.

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