Short review

The evolving role of Zebrafish (*Danio rerio*) in drug screening for lifestyle disorders: a narrative review

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Abstract

The aim of this review is to briefly illustrate both the advantages and the limitations of using zebrafish as a model system for lifestyle based human diseases and drug discovery. The availability of a model predictive animal model of disease is one of the cornerstone in the drug discovery and development. In this context, bibliographic searches were performed under Pubmed, Medline and Google scholar for articles indexed in these databases ranging from 2001 to 2021. Zebrafish has evolved as a suitable animal model organism for experimental pharmacology is expanding at a great rate to include lifestyle diseases of human. Lifestyle diseases are characterized by conditions which occur in primarily based on the way of living and occupational habits of individuals. Diseases that impact on our daily lifestyle are mainly obesity & diabetes. Nowadays, diabetes & obesity are considered as global epidemics. The prevalence rates of diabetes are increasing day by day in parallel with the rates of obesity. Research is ongoing for surgical and pathological management of obesity and diabetes. For identification and development of effective treatment the use of animal models are important. Zebrafish is poised to present as a unique model for human disease. In this study we discuss the advantages, disadvantages of pathology associated with diabetes & obesity by using zebrafish model. In consideration of these potential shortcomings, it is expected that zebrafish will not replace the classical mammalian test systems anytime soon, but rather complement them as a first step in vertebrate modelling of disease and aid the complex process of drug discovery.

Keywords: *Danio rerio*, Lifestyle disorder, screening, pharmacology

Introduction

Leishmaniasis is composed of group of diseases caused by Zebrafish is a well-established and powerful model for the study of vertebrate biology. Scientifically zebrafish is known as *Danio rerio* which is belonging to the family Cyprinidae of the order Cypriniformes. Zebrafish is a vertebrate and having a high degree of physiological, anatomical and genetically based similarities to humans. It is reported that the organism shares 70% of the human genotype as also with 84% of genes are known to be associated with human disease.

Zebrafish has the capacity to produce hundreds of offsprings in a week; they grow at extremely fast rate. Zebrafish has a short reproductive cycle and it is suitable for large scale drug screening. Zebrafish acquire unique characteristics that make this tropical fish a convenient animal model for developmental and genetic studies a follows:

I. This model organism reaches sexual maturity in about 2-3 months.
II. The small size of this model allows for cultivating relatively large numbers in small area.
III. Female Zebrafish are very fecund and can produce hundreds of eggs on weekly basis.

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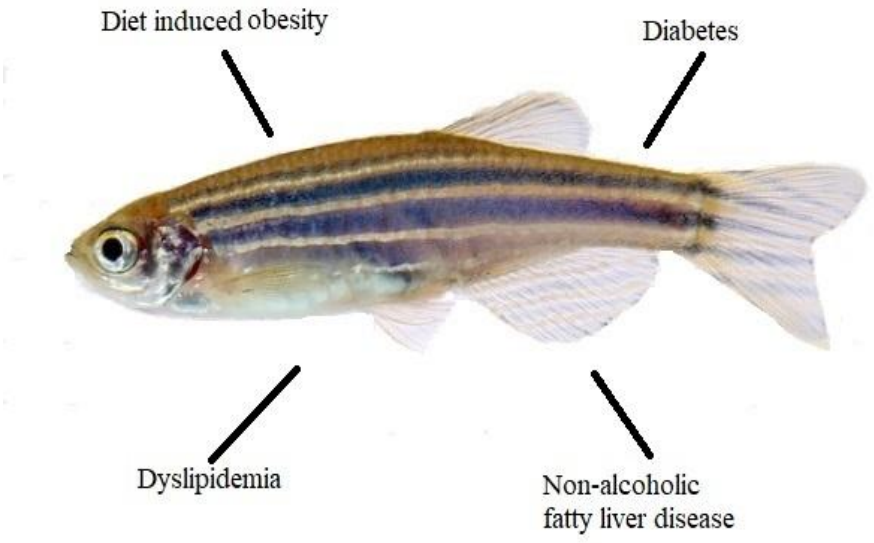

Fig 1: Representative of applications of Zebrafish (*Danio rerio*) models and its possible applications in studying human disease

IV. Egg fertilization process occurs externally which allows for the production of haploid embryos.
V. The growing embryos are transparent.
VI. Water soluble drugs are rapidly administered to Zebrafish by adding them to water.
VII. Zebrafish are susceptible to injection.[4]

Possible role in toxicology and Drug Discovery

Toxicological studies into Zebrafish have only recently emerged for augmenting drug discovery. Drug effects on growth and development have been assessed by gross visual examination of the length and shape of the body segments of *Danio rerio*, the size and morphology of internal organs, including the brain, liver, cardiovascular system, cartilage, notochord, pancreas, intestine, and kidney. In addition, organ function assays have been and continue to be developed, which permit functional assessment of drug effects on the major internal organs in vivo. A large number of Phase I oxidation enzymes (e.g. the cytochrome P-450 family), Phase II conjugation can be studied on zebrafish.

What makes Zebrafish such a predictive animal model [3]

Zebrafish matches all the criteria in the selection process of the animal model that directly related to the final goal. Zebrafish are attractive animal models as they have numerous advantages over other species. The most beneficial features of Zebrafish are the presence of a fully sequenced genome, easy manipulation of genome, high rate of productivity, external fertilization, rapid embryonic development, short generation time and transparent embryo. Zebrafish have all the main useful organs involved in the metabolic process which is beneficial to study various human metabolic disorders like diabetes mellitus, obesity, hepatic disease, dyslipidemia, non-alcoholic fatty liver diseases, etc.

Zebrafish models for diet-induced obesity [2]:

The metabolic rate of Zebrafish is not regulated by environmental temperature as it is an ectothermic species. Zebrafish have numerous adipose tissue depots and doesn't have brown adipocyte tissue (BAT) depots. At the very first stage, chicken egg yolk solutions, heavy cream are used as a high-fat diet for Zebrafish larvae and juveniles. This diet rapidly increases adiposity in Zebrafish. Adult Zebrafish are also used for obesity models.

Adult fish are routinely fed with 60mg *Artemia* per day for 8 weeks. After overfeeding, they exhibited hypertriglyceridemia, increased the rate of BMI and hepatosteatosis compared to normal Zebrafish. Thoroughly Zebrafish is a very alluring model system to evaluate the effects of compounds and foods on obesity.

The diet-induced obesity approach helps us to understand the disease of systemic obesity and therefore mimicking the common process occurring in humans affected by this same condition has utility in clinical medicine and pharmacology.

Zebrafish and a diabetes model [2]**:**

Zebrafish is a proper model for studying metabolic dysfunction because they have appropriate organs that involve metabolism including increased adipose tissue, cardiovascular overload, steatosis, and energy homeostasis. The preservation of the structure of the pancreas and glucose homeostasis system make Zebrafish convenient to identify novel targets in pancreas related diseases like type I & type II diabetes mellitus. Basic cellular architecture & morphogenesis of Zebrafish pancreas is identical with mammalian pancreas which has both exocrine & endocrine compartments. Zebrafish pancreas function has been well-established by some methods which include fasting & postprandial glucose measurements and intra-peritoneal glucose tolerance tests and techniques for islet cell culture and pancreas dissection.

Table 1 summarises current information of the models of Obesity using Zebrafish

Type of model	Induction strategy	Age at induction	Characteristics
High-fat diet	i) Heavy whipping cream	Larvae	Lipid accumulation inter segmental vessels; increased whole larval triacylglycerol(TAG) & apoliprotein B levels
	ii) chicken egg-yolk	Larvae, juvenile and adult	Hyperlipidemia, increased adipose tissue area & TAG
	iii) corn oil & lard	Adult	Increased body fat
Over-nutrition	*Artemia*	Adult	increased rate of BMI and hepatosteatosis, hypertriglyceridemia
Over-nutrition & High-fat diet	i) Tetramin & vegetable oil	Juvenile and adult	Increased weight gain & cardiovascular overload
	ii) artemia& egg yolk powder	Adult	Increased body weight, adipose tissue mass, adipocyte hypertrophy, Hyperglycemia, hepatosteatosis

Zebrafish model for dyslipidemia [3]**:**

Abnormal level of lipids (cholesterol, triglycerides and fat phospholipids)in blood results dyslipidemia which can lead to the development of atherosclerosis. Human atherosclerosis symptoms are very similar to the histopathological changes showed by overfed Zebrafish with a high level of cholesterol.

Zebrafish model for non-alcoholic fatty liver disease [3]

It is mainly build-up of extra fat in liver cells which do not related to overconsumption of alcohol and can lead to fibrosis, cirrhosis, steatosis, hepatocellular carcinoma, and steato-hepatitis. In association with insulin resistance, this disease can develop. These mechanisms are quite similar to humans. The human liver resembles the Zebrafish liver in function, genetics and cellular structure. This assertion led to study broad embryological and genetics which associated with the human liver disorder as well as potential therapies of liver disease. It was showed in an experiment, that when a Zebrafish was immersed in a 6% fructose solution; as a result, there is a formation of hepatic steatosis as similar to the symptoms shown in humans fed with a high-carbohydrate diet.

Limitations of this model

There is dissimilarity of organs like the reproductive system & respiratory system. Thus there is limitation to use Zebrafish as a model for reproduction & respiration in humans. On the other hand, the screening of some water-soluble drugs in Zebrafish is another limitation as they live in an aquatic habitat. Consequently, Zebrafish lack some of the important mammalian organs such as lung, skin, and mammary gland which limit its possible use in mimicking certain groups of human disorders. More generally, the closer the organism is on the evolutionary tree to the humans, the better the predictive value of the animal model. Yet, even nonhuman primates are not entirely predictive for human outcomes in drug development. Thus, the utility of the Zebrafish system needs to be carefully validated in the context of drug discovery programs. In consideration of these potential shortcomings, it is expected that Zebrafish will not replace the classical mammalian test systems anytime soon, but rather complement them as a first step in vertebrate modelling of disease and aid the complex process of drug discovery.

Conclusion

From the aforesaid summary we conclude that the use of adult or larval forms of Zebrafish serves as relevant model of considerable potential in fields of developmental biology and medicine. Zebrafish have several convenient features with respect to physiological, developmental and genetic studies

Table 2 summarises current information of the models of diabetes mellitus using Zebrafish

Type of model	Disease type	Induced by	Age	Phenotype
Pancreatectomy	T1DM	Physical removal of pancreas	adult	Elevated blood glucose level
Chemical ablation of β cells	T1DM	Intraperitoneal injection of streptozotocin(STZ)	adult	Hyperglycemia and diabetic complications
Glucose intolerance	T1DM	Alloxan exposure through incubation /IP injection	Larva, adult	β cells necrosis, decreased neuromast number
Glucose immersion	T2DM	Incubation in glucose solution	adult	Hyperglycemia, impaired response to insulin, diabetic retinopathy
Over-nutrition	T2DM & obesity	Over feeding zebrafish with commercial foods	adult	Hyperglycemia, glucose intolerance, insulin resistance

including transparent nature of embryo, external fertilization. There are enormous conservations of genetics, physiology and morphology between human and Zebrafish makes it an interesting model for various human disorders and development of potential therapies. Advancements of molecular technologies and nanotechnologies also contributes to the use of Zebrafish to learn different diseases in humans.in this review article we emphasized some areas where Zebrafish are an attractive model to investigate the processes and mechanisms which are associated with metabolic disorders like diabetes mellitus, obesity, liver-related disease, dyslipidemia, atherosclerosis, and intestinal disease. Recently, Zebrafish have been also used by scientist to develop new therapies for treatment and prophylaxis of various non-communicable human diseases.

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Conflict of interest
None

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