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Review Article

ZEBRA FISH: AN EXPERIMENTAL MODEL FOR DRUG SCREENING

Mahesh B Narkhede*, Pavan P Chinchole, Mangesh N Deokar, Kiran P Gaikwad, Ananta G Titare, Sonali P Mahajan, Nikhil G Ratnaparkhi

Department of Pharmacology, Dr Rajendra Gode College of Pharmacy, Malkapur Dist Buldana, Maharashtra, India
**Corresponding author: maheshnark@gmail.com*

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ABSTRACT

Animal models have provided invaluable information in biological evaluation and drug screening. It comes to predicting the human body's response to candidate drugs; traditional laboratory animal models are woefully inadequate. However, increased concern for the welfare of the animals used, and a growing awareness of the concept of animal rights, has brought a greater focus on the related ethical issues.In this review, we seek to summarize the important anatomical aspects of Zebra fish as it is used as an alternative animal model for drug screening.

Keywords: Zebra fish, Animal model, Drug screening.

1. INTRODUCTION

Animals have been used in studies and research for eras in human history. The use of animals for scientific purpose is both an ancient practice in biological research and medicine, and a frequent matter of debate in our societies. The remarkable anatomical, physiological, pathological and pharmacological similarities between humans and animals prompted researchers to investigate large range of mechanisms and assess novel therapies in animal model before applying to the humans [1]. The use of animals is not only based on vast commonalities in the biology of most mammals, but also that human diseases often affect other animal species. Some animals have a short life span (fish: 13 weeks; rat: 12-2 years, rabbit: 1-2 years) which makes it suitable to be studied throughout their entire life. The environment can be easily controlled to keep the experimental variables constant. By using animals, researchers can carry out experiments that would be impractical or ethically prohibited with humans. In some cases, there are around 99% genetic similarities between animals and humans [1].

Humans and other mammals are very complex organisms in which organs achieve distinct physiological functions in a highly integrated and regulated fashion. Animal models have greatly improved our understanding of the pathophysiology and have provided a valuable platform for testing potential therapeutic strategies. Animals also help us to understand the biological functions of the genes and their proteins and how defects result in the disease condition. Hence, animals' models for human disorders have contributed immensely on the pathophysiological processes and on the normal cellular functions of the gene implicated.

The Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) is an authority which monitors animal experiments conducted in institutions through ethics committee and is mainly concerned with promoting the human care of animals used in biomedical and behavioural research [2]. For this purpose, the Government has made "Breeding of and Experiment on Animals (Control and Supervision) Rule 1998" as amended during 2001 and 2006, to regulate the experimentation on animal [3].

The Zebra fish (*Danio rerio*) is a freshwater fish belonging to the minnow family (Cyprinidae). It has the distinctive horizontal strips extending across the length of its entire body. It originates from the eastern India and inhabits bodies of fresh water such as streams, ponds, and canals. It is a popular aquarium fish, frequently sold under trade name Zebra danio [4] (and thus often called a tropical fish although both tropical and subtropical). It is around 5-6 centimetres long [5] and can live for up to 5 years (3 years average) [6, 7].

Fig. 1: Zebra fish as a model organism for drug screening

2. ANATOMY AND PHYSIOLOGY OF ZEBRA-FISH

2.1. Heart

Apart from sharing well-conserved genetic pathways that govern heart formation, zebrafish heart development proceeds through very similar steps as amniotes (vertebrates). In the zebrafish, the heart is situated anterior of the main body cavity and ventral to the oesophagus [8, 9]. Zebrafish heart is composed of 4 chambers (sinus venosus, atrium, ventricle, and bulbus arteriosus) connected in series, one atrioventricular (AV) valve, and one ventricular- bulbar valve (outflow valve) [10, 11]. Heart valves composed of leaflets ensure unidirectional blood flow in zebrafish. One valve that is AV valve is situated between atrium and ventricle, whereas another valve that is ventricular bulbar valve between ventricle and bulbus arteriosus [11].

Deoxygenated venous blood enters the sinus venosus. The wall of the sinus venosus is thin and is mainly composed of collagenous connective tissue. Contraction of the atrium and dilation of the ventricle forces the blood into the ventricle via the atrioventricular valve. There is a compact outer layer of muscle and a spongy inner layer with numerous trabeculae. Because of its elasticity, it can inflate considerably, thereby reducing the ventricular pulse pressure [12].

The cardiovascular system appears when needs for oxygen and nutrition cannot be met by diffusion alone, because of the volume or increased metabolic rate of an organism during the course of embryogenesis [13, 14], the heart is the first definitive organ to develop and become functional, as any later survival depends on its proper function [15]. However there is a period when the heart is already functional, but not yet essential in the early developmental stages.

Zebrafish heart development proceeds through analogous steps with other vertebrates [16]. Gastrulation is completed at 10 hpf, followed by the fusion of bilateral heart fields at the embryonic midline at 16 hpf. This results in the formation of a cardiac cone, which then extends anteriorly and transforms into a linear heart tube [17, 18]. The outer layer of the linear heart tube is composed of endocardial cells. The two layers are separated by an acellular extracellular matrix layer known as cardiac jelly. Following its development, the linear heart tube starts to contract in a rhythmic peristaltic manner at 24 hpf [18].

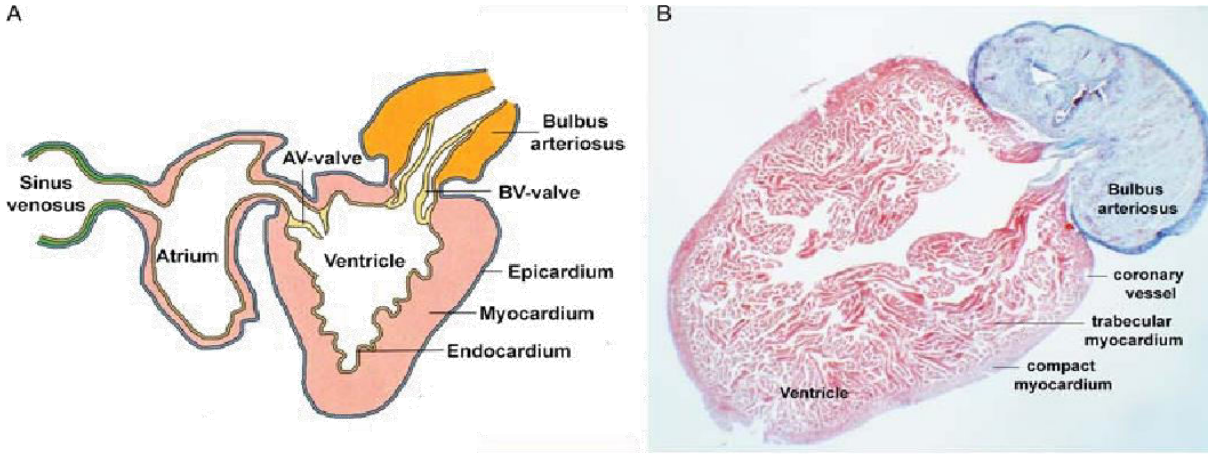

Fig. 2: Zebrafish heart chambers (sinus venosus, atrium, ventricle, bulbus arteriosus) and valves (AV-valve and BV-valve) composed of different layers which include epicardium, myocardium, and endocardium[13]

2.2. Digestive system

Previous studies have shown that intestinal anatomy and architecture in cyprinid teleost fish is closely related to mammals [19, 20]. The intestine of the adult zebrafish consists of one long tube that folds twice in the abdominal cavity and thus inhabits the majority of the abdominal cavity. Proportionately, these folds are significantly larger than the finger-like intestinal villi of mammals and other amniotes [21]. Many folds are oriented circumferentially, but a substantial percentage of folds are randomly organized. Fold height is smaller in the mid vs. anterior intestine. The shortest folds that are oriented longitudinally define the posterior intestinal segment [21].

Three of the four principal cell types within the mammalian small intestinal epithelium are present within the anterior zebrafish intestine. They are- Columnar-shaped absorptive enterocytes [22] Goblet cells, Entero-endocrine cells [23]. A schematic diagram comparing the structure of the adult zebrafish and mammalian intestine is depicted in fig.3.

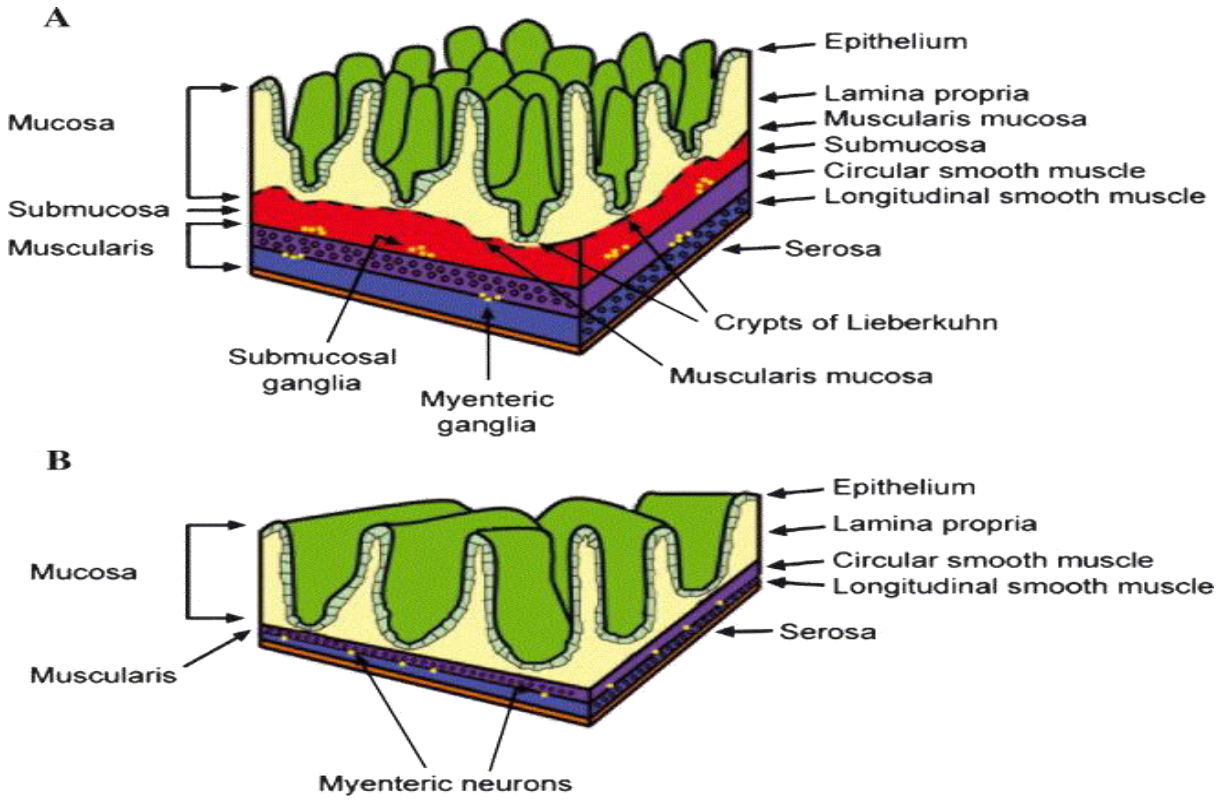

Fig. 3: Difference between the mammalian and teleost intestinal structure, illustrates intestinal layers within the mammalian (A) and teleost (B) intestine [24]

2.3. Brain anatomy

The zebrafish brain consists of different parts, which are described as follows:

Spinal Cord: The neurons projecting fromthe brain to the spinal cord as well as the terminal fields of ascending spinal projections in the brain of adult zebra fish with unlesioned or transected spinal cords. Twenty distinct brain nuclei were found to project to the spinal cord. These nuclei were similar to those found in the closely related goldfish, except that additionally the parvocellular preoptic nucleus, the medial octavolateralis nucleus, and the nucleus tangentialis, but not the facial lobe, projected to the spinal cord in zebrafish [25].

Cerebellum*:* The cerebellum functions in the control of smooth and skillful movements. It is also implicated in a variety of cognitive and emotional functions the cerebellum integrates sensory and predictive inputs, which include proprioception and information associated with motor commands, to elicit precise motor control and higher cognitive/emotional functions. These complex tasks rely on the well-organized structure of the cerebellum and neural circuits [26].

Thalamus: Current research on the thalamus and related structures in the zebrafish diencephalon identifies an increasing number of both neurological structures and ontogenetic processes as evolutionary conserved between teleosts and mammals. A case in point is the migrated preglomerular complex of zebrafish which evolved only within the lineage of ray-finned fish and has no counterpart in mammals or tetra pod vertebrates [27].

Neurogenesis describes the process by which undifferentiated neural progenitor cells generate mature and functional neurons. The main architecture of the systems in zebrafish brain resembles that of the mammals, despite differences in the development of the telencephalon and mesodiencephalon. Modulatory neurotransmitters systems which degenerate in human diseases include dopamine, noradrenaline, serotonin, histamine, acetylcholone and orexin/hypocretin. Although the number of G protein-coupled receptors in zebrafish is clearly larger than in mammals, many receptors have similar expression patterns, binding and signaling properties as in mammals. Distinct differences between mammals and zebrafish include duplication of the tyrosine hydroxylase gene in zebrafish, and presence of one instead of two monoamine oxidase genes. Zebrafish are sensitive to neurotoxins including MPTP, and exposure to this neurotoxin induces a decline in dopamine content and number of detectable tyrosine hydroxylase immunoreactive neurons in distinct nuclei. Sensitivity to important neurotoxins, many available genetic methods, rapid development and large-scale quantitative behavioral methods in addition to advanced quantitative anatomical methods render zebrafish an optimal organism for studies on disease mechanisms [28].

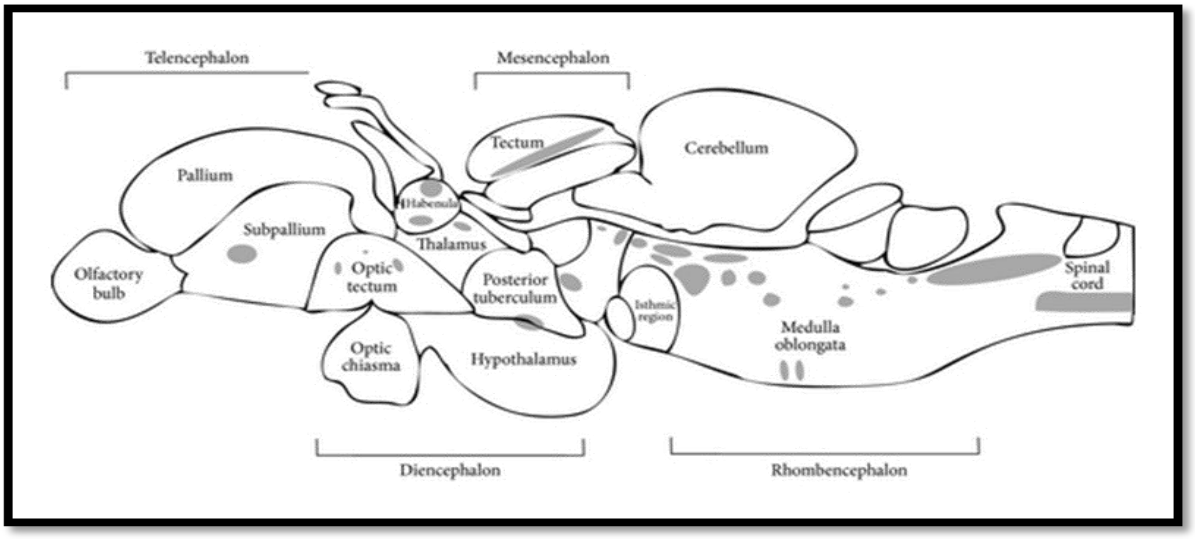

Fig. 4: Zebrafish brain: This schematic diagram depicts the different parts of the brain [29, 30]

3. EXPERIMENTAL MODEL

The zebrafish has become known as an excellent model organism for studies of vertebrate biology, vertebrate genetics, embryonal development, diseases and drug screening [31]. Intraperitoneal and intracere-broventricular injections, blood sampling and measurement of food intake are possible to be carrying out in adult zebrafish. It is a useful animal model for neurobiology, developmental biology, drug research, virology, microbiology and genetics. A lot of diseases, for which the zebrafish is a perfect model organism, affect aquatic animals [31]. Cardiovascular studies in zebrafish have primarily focused on the embryonic heart. Studies using zebrafish cardiac mutants and morphants have revealed insights into cellular and molecular mechanisms regulating embryonic cardiac contractility and rhythmicity [32]. One remaining question is whether the fish model could be utilized for investigating adult cardiac disorders. Recent studies showed that adult fish heterozygous for the KCNH2 mutation have prolonged QT interval similar to the dominant trait observed in humans [33].

The zebrafish model offers several advantages in examining the genetic mechanisms behind cardiovascular development and function. First, zebrafish produce a large number of easily accessible and

transparent embryos, facilitating phenotypic driven genetic screens. Second, the embryonic zebrafish heart is placed at a prominent position at the ventral side of the embryo allowing visual inspection of the patterning and function of the developing heart in live embryos [32]. zebrafish hearts maintain their ability to regenerate throughout their lifetime, providing novel insights to understand human cardiac regeneration.

Zebrafish (*Daniorerio*) has been a prominent model vertebrate in a variety of biological disciplines. Substantial information gathered from developmental and genetic research, together with near-completion of the zebrafish genome project, has placed zebrafish in an attractive position for use as a toxicological model. Although still in its infancy, there is a clear potential for zebrafish to provide valuable new insights into chemical toxicity, drug discovery, and human disease using recent advances in forward and reverse genetic techniques coupled with large-scale, high-throughput screening. Here we present an overview of the rapidly increasing use of zebrafish in toxicology. Advantages of the zebrafish both in identifying endpoints of toxicity and in elucidating mechanisms of toxicity are highlighted [34].

4. DISCUSSION AND CONCLUSION

Zebra fish is used from eras in scientific research and development. Some of the scientists use zebrafish as an experimental model for different purposes. The use of zebrafish as a model organism was pioneered by George Streisinger in 1970 at University of Oregon, USA. He is the "Founder Father" of Zebrafish Development and Genetic Research. In 1990, the first large scale mutagenesis of Zebrafish were conducted by Christiane Nusslein Volhard in Oxford University, United Kingdom to identify developmental mutations. Thomas Look of Dana-Farber Cancer Institute, Boston uses the translucent zebrafish to study how cancer behaves in 1995. Zebra fish animal models are being used for experimental studies in various branches of medical sciences such as cardiovascular, neurology, toxicology, organ transplantation and regeneration. Zebrafish have been used predominantly in developmental biology and molecular genetics, but their value in toxicology as well as drug discovery has been recognized.

Conflict of interest
None declared

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