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Review Article

ZEBRAFISH (*Danio rerio*): THE FUTURE OF ANIMAL MODEL IN BIOMEDICAL RESEARCH

Rajla Bressan Simonetti[1*], Lis Santos Marques[2], Danilo Pedro Streit Jr[3], Eneder Rosana Oberst[4]

[1]Veterinarian, UFRGS, Porto Alegre, Brazil

[2]Animal Science Faculty, UFRGS, Porto Alegre, Brazil

[3]Animal Science Faculty, UFRGS, Porto Alegre, Brazil

[4]Department of Veterinary Clinical Pathology, Faculty of Veterinary, UFRGS, Porto Alegre, Brazil

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Abstract: The zebrafish (*Danio rerio*) has achieved high popularity as a model organism of favorable characteristics founded on a unique set of properties as high fecundity, small size, rapid development, and rapid generation time. Although the husbandry of this species is poorly developed in contrast to the scientific rigor that is required, this species has great potential as an experimental model, from embryology due to the optical clarity of the embryos and larvae, extracorporeal development of the embryos, the studies of brain disorders by the high similarity of the nervous system between zebrafish and human. In addition, this manuscript discusses new lines of research in various biomedical areas, emphasizing the biological importance for the research of the species.

Keywords: Zebrafish; Animal experimentation; Biomedical research.

[*]Correspondence to: **Rajla Bressan Simonetti Veterinarian, Department of Veterinary Clinical Pathology, Faculty of Veterinary, UFRGS, Porto Alegre, Brazil**

E-mail: rajla_s@hotmail.com **Tel:+55 51 98339216.**

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Introduction

Animal experimentation has an important role in scientific evolution. Although some models have been replaced by alternative methods, scientific research still needs animal models for development, reliability and legitimacy of science. The use of zebrafish (*Danio rerio*) as an experimental model has grown rapidly for biomedical research. When thinking about medical research, these animal models probably are not the first animals that come to mind. However, researchers worldwide are increasingly interested in this small tropical freshwater fish, and one of the most widespread reason is that embryos of this species, unlike the mice, develop outside the mother's body and are transparent. This is a great advantage that allows researchers to study in detail the vertebrate embryonic development without the need for invasive procedures. Another beneficial factor is that zebra fish can produce 200 to 300 fertilized eggs weekly and complete the embryogenesis in 72 hours. And yet, adults and embryos of this species are small in size, have lower cost, and the generation interval is short.

Howe et al. (2013) divulged the sequence of the zebrafish genome, revealed that these fishes, mice, and humans have 12719 common genes, so 70% of human genes are found in *Danio rerio*. Thus, when the genes that cause diseases in humans are injected in zebrafish embryos, the fish in growth eventually acquire the same disease. For this reason, the species has been widely used in studies to aim the cure of genetic disorders, such as depression, schizophrenia and Parkinson's disease. In addition, various aspects of the physiology and morphology resembles that of human, such as muscle fibers, central nervous system, skeletal system complex, multiple hematopoietic cells (erythrocytes, myeloidcells, Band T lymphocytes, etc.), and cardiovascular system (Lieschke;Currie, 2007).

Biology

The zebrafish (Daniorerio) is atropical fresh water teleostei, belonging to the Cyprinidae family. This species is characterized by small size, adult measures about 4-5 cm, have a cylindrical body, and a distinct color pattern alternating light and dark horizontal stripes (Spence et al., 2008). Exhibit sexual dimorphism: males are thinner and generally golden in the ventral region, females are more rounded and silvery, mainly in the ventral region, which is most evident in the period close to the spawn. Females may spawn every 2-3 days and a single spawn may contain hundred eggs. The spawn may contain 200 eggs from a single female; the fry grow quickly and can reach sexual maturity within 2-3 months (Gerlai et al., 2000).

Geographic Region

The natural habitat of the zebrafish is in South Asia, Ganges and Brahmaputra rivers basins, in northeastern India, Bangladesh, Nepal and northern Myanmar (Spence et al., 2006). The south of Asia is characterized by a long rainy season, with a wide seasonal variation: rainy and drought season, well defined, constantly varying the aquatic habitats extensions (Spence et al., 2007). The rivers in this region continually change through the seasons, leaving the lakes and canals beel, changing the formation of lakes and marshes due to flooding, especially during the monsoon season (Spence et al., 2006). These standing water paths influence habitat parameters, including water chemistry and its abundance, and consequently the biology of zebrafish, breeding, feeding, and development. The results of Spence et al. (2006), *Danio rerio* were the most commonly found in non-saline areas of lowland. Studies performed in wild and domestic Zebrafish indicated that both prefer to perform spawning in locations associated with the submerged aquatic vegetation (Spence et al., 2007). The preference is standing or slow moving water, slightly alkaline (pH ~8.0), and relatively high clarity.

Water Quality

The water quality parameters for its breeding in captivity are well established (Westerfield, 2000). However, it is important to note that the quality of water as well as the feeding has a major impact on the survival and reproduction of the zebra fish. On the other hand, one of the reasons for the rise of the zebrafish as an experimental model is the tolerance of the environmental conditions in captivity and for its adaptability, and it is a reflection of its distribution in the environment. However, we must recognize that there is an energy cost of the fish to operate out of their comfort range.

Engeszer et al.'s research (2007), majority of the zebra fish collected from the nature were found in environments with pH between 5.9-8.1. High concentrations of amnonia or nitrite can cause damage to the fishes, nitrite is absorbed through the gills and interfere with the capability of the fish to absorb oxygen (Reed; Jennings, 2010).

The zebrafish are eurythermic, which means that they can tolerate a wide range of temperature. López-Olmeda; Sánchez-Vázquez (2011) collected animals from the nature, in places with temperature varying from 16.5°C to 38.6°C. The temperature influences the hatching rate of embryos, in low temperatures (24-26°C), the hatch will take longer than in higher temperatures (28-30°C). The water quality parameters are vital to the production in captivity, because it minimize mortality and increase the number of high quality embryos (Lawrence, 2007).

Diet

The zebrafish are omnivores, have no true stomach and possess a long intestine with a large absorption area (Ulloa et al., 2011). As a gastric species, it is speculated that the fish would perform better when fed small meals during the day (Lawrence, 2007). In the nature, the zebra fish dietary components are zooplankton and insects, even though phytoplankton, filamentous algae and vascular plants have been found in the digestive system, well as eggs, probably small crustaceans. The insects were mainly dipterous (Spence et al., 2007).

Due to this tendency to consume larvae of dipterous, it was proposed the use of zebrafish in the control of mosquitoes (Lawrence, 2007). The feeding once a day appears to be sufficient to support the growth and reproductive performance for this species. The frequent feeding will create heavier females, however it does not translate to higher growth rate, suggesting that a feed per day is sufficient in juvenille state. Information on food preference of wild zebrafish are relevant for handling, once they are decisive in the successful creation in the laboratory. For the survival and increase growth and reproduction, the nutrients requirements should be established and applied in captivity.

Reproduction

The fertilization and the developement of the zebrafish are external. The female are asynchronous, that is, the spawing occurs multiple times a day. The reproduction is done in small groups, spreading the eggs beneath the ground, without any parental care (Lawrence, 2007). The sexual maturity is reached at around 10-12 weeks of life; however, it is advisible that the reproduction starts at six months, to obtain a good reproduction performance and embryos with better quality (GODOY, 2012).

Behavior

The zebrafish has been used in normal behavioral studies, as well as in pathalogical, (Stewart et al., 2011) and in brain disorder studies (Kalueff; Stewart; Gerlai, 2014b; Stewart et al., 2014b). Therefore, knowing the phenotypic behavior of the zebrafish (Jia; Fernandes; Gerlai, 2014) is important to comprehend neural pathways, physiological biomarkers, and genetic basis of normal and pathological brain function of this kind. Since there is no standardized terminology, the recognition of the zebrafish behavioral phenotypes is still a challenge. In order to improve the interpretation of published results, it has been developed a comprehensive catalog of zebrafish behavior, the ZBC (Zebrafish Behavior Catalog) that covers both adult models as larvae (Kalueff et al., 2013). The ZBC create daglossary that cites the specific behaviors, referring to them by numbers, standardizing the identification of zebrafish behavior (Kalueff et al., 2013). There still is the technique of measurement of phenotypes with specialized software video for traceability (Egan et al., 2009; Canavello et al., 2010). The Dabio rerio is often described as a fish with shallow water habits, even if they swim near the bottom during oviposition (Spence; Smith, 2005) and during the procession use all the water column (Spence et al., 2006). In the experiment of Gerlai et al., (2000), the zebrafish preferred the bright environment rather than the dark environment. This species often swim in small shoals (5-20 individuals) in standing water and prefer to associate with the most active schools (Pritchard et al., 2001). By presenting a series of social behavior, such as territoriality and social learning the zebrafish often exhibit agonist behavior, fight or escape, (Ricci et al., 2013). The aggressions, by biting or persecution, occurs mainly during the establishment of hierarchies and mating. Males are territorial around potential sites of oviposition (Spence; Smith, 2005), and can also adopt the persecution tactics to females (Goolish et al., 1998 apud Lawrence, 2007). Females choose their partners (Spence; Smit H, 2005) by factors related to the physical, visual interaction, and olfactorycues (pheromones) thatcauseacascadeofhormonalresponsesthatinfluence maturity and breeding. Gerlach (2006) reported that females exposed to male odors produced 17% more viable eggs than the unexposed females. Gerlach; Lysiak (2006) suggest that schools of larvae in nature are preferably closer to relatives. The degree of genetic relatedness of individuals in adult schools is unknown, although there is experimental evidence indicating that the preference of the individual to join brothers changes after sexual maturity, suggesting that somewhere in the larval or juvenile fish life, it is dispersed away from their home shoal (Gerlach; Lysiak, 2006). This premise is supported by genetic studies collected at four different sites in India that have shown that populations high levels of genetic variability and low genetic structure between fish (Lawrence, 2007).

Lineages

The zebrafish can be purchased at pet stores or simply caught in the wild, however, according to Vignet et al. (2013) there are differences in behavior between wild strains, therefore, the lineage to be used should be appropriate to the objective of the experiment. The lineage of *Danio rerio* most used are AB e TU (Tübingen). The lineage AB, developed by George Streisinger, is an active lineage, commonly used in genetic manipulation and pharmacological tests, and the TU, developed by the Tübingen Group, it has shorter fins; and it is commonly used in behavioral research. In the Zebrafish Database 1 are registered 26 different strains of wild type. Only AB and TU strains are genetically defined (Vignet et al., 2013). The lineages for color and phenotype: pink, leopard (dark spots), albinoandlongfin, these last three by spontaneous mutation (Kalueff; Stewart; Gerlai, 2014b). Several studies comparing wild lineages with domesticated, showed behavioral differences that exist between them. Drew et al. (2012) observed that the domesticated strain spent more time near the water surface and occupied more the front of the tank, in the presence of human. Furthermore, Vignet et al. (2013) suggested that TU lineage is more Disponível em www.zfin.org. Acesso em: 17 jul. 2014. active than the AB line, as this presented more anxious behavior and preferred the shade, this explains why such a strain is normally not used in behavioral studies. Inlaboratory strains, It has been observed rapid growth rate, increased sexual dimorphism reduction behavior pattern to avoid predators and a greater degree of orientation in the surface, when compared with the wild strain in India (Reed; Jennings, 2010).

Animal experimentation

Biomedical research relies on the use of animal models to understand the pathogenesis of human and animal diseases to develop and test new therapies (Lieschke; Currie, 2007). A number of factors must be considered for the use of experimental models; in additiontosignificantevolutionaryproximityandanatomicalsimilarities,it should be considered the approach of cellular processes (Lieschke; Currie, 2007). The use of animals in scientific research has generated great discussions in recent years, and there has been growing concerns related to bioethics and animal welfare. One of the arguments raised in the debates is that alternative methods could replace the use of animals in biomedical research. In the scientific community it has been growing interest in alternative methods aimed at reducing the use of animals in experiments, as these represent a high cost in research, since they must be contained, fed and kept healthy for scientific purpose. Alternative methods correspond approximately 90% of biomedical research (Medical..., 2007) and include mathematical and computational models, advanced tissue and cell cultures, and scanning technology.

History in Biomedical Research

The *Danio rerio* was first described by Francis Hamilton, a surgeon from British East India company, established in West Bengal, India, in the early XIX century. He published An account of the fishes found in the river Ganges and its branches in 1822, among the fishes where ten copies of the zebrafish (Spence et al., 2008). Initially it was called Brachy*danio rerio* until its transfer to the genre *Danio rerio*. Development and embryology (Lieschke;Currie,2007). In 1934, Charles Creaser published an article describing the handling of the zebrafish and emphasizing the ease of studies of this species. George Streisinger began working with zebrafish in the late 1960s (Grunwald; Eisen, 2002), and in 1981, a publication of the group led by Streisinger at the University of Oregon, suggested that one could apply a mutational analysis in embryonic development, producing haploid embryos from eggs treated with ultraviolet radiation. From there it began a search not only in the development mutagenesis embryonic, as well as molecular genetic later neuroscience, toxicology, tissue regeneration, ethology, among other areas of interest. The main reason for Streisinger to choose the zebrafish as experimental animals was the need to move closer to human models; invertebrates, which were being widely studied in his day, for a vertebrate model. In addition, the need for models for genetics that were of small size, rapid reproduction, and wide progeny (MEUNIER, 2012).

Advantages and Disadvantages of Zebrafish in Biomedical Research

The *Danio rerio* has emerged as an excellent model for research in different areas, mong them pharmacogenetics, neurology and embryology. The main reasons are the easy access to all stages of development, the optical clarity of embryos and larvae that allows view in greal-time development, in addition to high genetic, easily manipulated and homologous physiology with humans, especially in the central nervous system. Its small size enables high storage, does not need large infrastructure facilities, as in mouse animal houses. The cost of using zebrafish is less than the mouse; per year, creating mice is about three times larger compared to the zebrafish (Lieschke; Currie, 2007). The generation time of the species is short (three months), and you can create many copies in a limited space (100 adults in a tank of 8-12 L) (Dammsky et al., 2011). Another advantage is the rapid reproduction and abundant (for a single spawn, the female produces about 100 eggs), and high (80-85%) homology to human gene (Howe et al., 2013). Thetransparencyofthezebrafishembryofacilitates-thestudiesingeneticdevelopment programs, because it is possible to monitor and manipulate its development without difficulties. Furthermore, rapid development shows, breaks in less than three days and become mature in 90 days, which makes the search more rapid. Among the various advantages of toxicology studies in zebrafish, some already mentioned, and apart from these, the embryos can live a few days after fertilization without external power. The drug readily diffuse across the skin and gills and penetrate orally after 72 hours after fertilization, not requiring active application of drugs (Lantz-Mcpeak et al., 2014). Moreover, both the larvae and the adult zebrafish are used as templates, resulting in the expression "in a two models" (Kalueff; Stewart; Gerlai, 2014b). Lack of knowledge about the genetics of their lineages is a limitation of this experimental model. And still, the strains that are known are not standardized, in contrasted, species such as the mouse, the lines are already well established (Kalueff; Stewart; Gerlai, 2014b). However, based on analysis of the PubMed on zebrafish, Kalueff; Echevarria; Stewart (2014) found that the publications on this species grew 15% from 2004 to 2013.

Why Replace the Mammal Model?

Important factors have been discussed on the mouse model, widely used in biomedical research that led to major scientific advances. One of the factors discussed are the problems of stress in mice, a paradigm of the model itself (Kalueff; Wheaton; Murphy, 2007). These animals are easily stressed, so some human behaviors can not be played entirely under these conditions, or can not be trusted. Among the difficulties in using mammals, it can be mentioned the need for a large space for creation, the cost of creation and the complex handling. For example, to collect mouse embryos is to sacrifice the female, or in pharmacological studies must be applied in each animal individually, or by inhalation in which the environment must be sealed. As in fishes, the solution on the water reaches all individuals.

Applications in Biomedical Research: Neurology

The use of adult and larvae zebrafish in neuroscience has increased in recent decades because of the similarity of physiology and genetics to human, and the ease of handling and similarity of the central nervous system (Kalueff; Stewart; Gerlai, 2014b). The morphology of the brain of mammals and zebrafish is similar, including macro-organization of the brain (Kalueff; Stewart; Gerlai, 2014b). As in other vertebrates, the zebrafish central nervous system is formed outside the neural plate, in an ectodermal epithelium layer on the dorsal side of the embryo (DE Esch et al., 2012). Another example is the similarity habenula, epithalamus core located in the brain, which regulates the serotonin and dopamine (Okamoto; Agetsuma; Aizawa, 2012) which have close correlation with the neurochemical disorders of depression. Clinical depression is strongly correlated with genetic factors, environmental stress, and neurochemical disorders, and it seems to play a similar function phenotypes in zebrafish. The gene, gr-s357, of zebrafish, with a mutation of the glucocorticoid receptor demonstrates an increase in glucocorticoid levels that induces abnormal behavior, such as reduced mobility and smoothly, that resemble the phenotypes observed in human clinical depression (Ziv et al., 2013). Based on a research referencing the zebrafish as a highly social species, which prefers to swim in shoals, (Gerlai, 2014; Engeszer; Ryan; Parichy, 2004; Pritchard et al., 2001), it was considered that it would be possible to use it in studies of autism spectrum disorder, a dysfunction of human development that affects the ability of communication and socialization. Some drugs are clinically effective in some symptoms of autism, but do not reduce other symptoms. Forexample, risperidone, an antipsychotic, effective to treat irritability, it does not reduce the symptoms of social impairment and repetitive / obsessive behaviors in humans and appears to have similar effects in zebrafish; this indicates that there is high sensitivity to drugs relevant for the disease (Stewart et al., 2014b).

There are some zebrafish genes related neurodegenerative diseases in humans and manipulation of these genes that can facilitate the understanding of the genetic mechanisms causing the pathogenesis (Lee; Freeman, 2014). Regarding neurodegenerative diseases in humans, Lopes da Fonseca et al. (2013), for example, describe a zebrafish gene homologous to the transmembrane protein, ATP13A2, human that plays an important role in embryonic development. This protein has been associated with Parkinson's disease; mutations in TP13A2 were observed in several cases of Parkinson's disease (Crosiers et al., 2011). Two homologous genes have been found in the *Danio rerio* with the same functions of the human protein, neurogenesis in the embryo (Lopes Da Fonseca et al., 2013).

Toxicology

The development of toxicological studies in zebrafish have offered several advantages to the tracking of a large number of drug compounds and toxicological studies (Lantz- Mcpeak et al., 2014). In Germany, the zebrafish embryo was introduced to the ISO test (ISO 15088:2007) for toxicological testing of water, replacing the adult zebrafish (Yang et al.,2009). Since 2002, the test using zebra fish embryos for waste water treatment is mandatory (Braunbeck et al., 2005). In the work of Chen and others (2012), the neurotoxicity of Pb in zebrafish embryoschanged the behavior of individuals as adults, these results showed that exposure to Pb caused deficits in learning and memory, indicating that this exposure persists into adolescence and in adulthood and affects mental development. In the experiment of van den Brandhof; Montforts (2010) growth retardation was observed during the development of zebrafish embryos exposed to teratogens, occurring deformations on the yolk sac and tail. In a study on the cardiotoxicity of anthracyclines (widely used in chemotherapy treatments, but in the long run can cause cardiac toxicity), Han et al. (2014) suggest toxic effects on the developing of the zebrafish embryogenesis, and changes in gene expression and hatching.

Cardiovascular

The *Danio rerio* has also been used as a model for cardiovascular disease, congenital heart defects to arrhythmias and cardiomyopathies (Asnani;Peterson,2014). myloidosis is a disease in which bone marrow cells produce amyloid, an abnormal protein substance that accumulates in various organs, including the heart, causing damage to the heart muscle. Zebrafish treated with amyloidosis developed cardiac dysfunction and apoptosis of cells, contributing to premature mortality from day 2 after treatment and the peak was between days 5-7 (Mishra et al., 2013). The cardiac amyloidosis is characterized by a low systolic blood pressure, coinciding with the contractile dysfunction found in zebrafish, the same as in humans.

Regenerative Medicine

In laboratory it has been documented that the longevity of zebrafish may exceed five years (Gerhard et al., 2002). The collection of age data for the wild fishes populations facilitate comparisons of physiological ages between wild and laboratory, which would be useful in studies involving aging (Lawrence, 2007). The research of zebrafish has become a bridge between the cell and the biological development due to its rapid development. The gastrulation is completed within ten hours after fertilization, leading some to speculate that zebrafish can become an ideal animal model to study cellular and sub-cellular (Beis;Strainier,2006), including regenerative medicine. The zebrafish fully regenerates the heart muscle with in a few weeks(Poss; Wilson; Keating, 2002), therefore it has been used in studies on regeneration. This species, as a model for heart damage, provides a means to guide the search cell types and pathways that may assist in the recovery of regeneration of cardiac patients (Goessling; North, 2014). The zebrafish has a number of features that make it an excellent model for research on aging: many descendants and their short lifespan relative to other mammals (Gerhard, 2003), the zebrafish takes only 30-40 minutes to their embryo be observed and manipulated, 72 hours to become larvae and 90 days is an adult; the rapid growth rate coupled with its longevity is a possible relationship to research on aging (Gerhard, 2003). The combination of high growth rates and rapid transformation in an adult body has several negative correlations, such as reduced immune competence, depletion of energy reserves and decreased life expectancy (Inness; Metcalfe, 2008). It is suggested that increased oxidative stress and mitochondrial damage lipids during the first month of life zebrafish can determine the short-term life (Almaida-Págan; Lucas-Sánchez; Tocher, 2014).

Conclusion

The purpose of animal model usage is to understand a particular disease without causing risk to a human. Although the *in vitro* cell cultures tests are widely used, the results may not generate actual forecasts compared with in vivo results. The use of animal testing is still necessary. Search costs are high and the time is costly, especially in mammals, therefore, it has to be sought a new model of animal experiments, including invertebrates and fish. With the search for new experimental models to reduce, refine, and replace the use of animal models, it was found the zebra fish. The usefulness of this kind has excelled in biomedical research is at its genome close to the human being, extracorporeal embryo, rapid development, complex physiology, easily accessed organs, model space/cost efficient, small; These various aspects put the zebrafish as an animal model of great importance, but there is still much to discover about this species. To further advance the research with *Danio rerio*, it is necessary to put more efforts so that new information can flow to the understanding of biomedical research combined with the use of zebrafish.

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