ECRONICON OPEN ACCESS

EC ANAESTHESIA
Review Article

Anesthesiology, Anesthetics and Zebrafish (Danio Rerio). An Animal Model to Perform Basic Biomedical Research

Rafael A Vargas*

Faculty of Medicine, Antonio Narino University, Colombia

***Corresponding Author:** Rafael A Vargas, Faculty of Medicine, Antonio Narino University, Colombia.

Received: March 20, 2018; **Published:** May 14, 2018

Abstract

Basic biomedical research is a necessity in the field of health since many drugs, medical devices and procedures require preclinical trials to guarantee the benefit and safety of the patients in whom they will eventually be employed. However, there are limitations to perform this type of research due to the high investment that basic research demands, especially in the infrastructure and care that the animal models used require and that allow compliance with the bioethical standards that regulate basic research. An animal model of low cost and high versatility can encourage preclinical research, especially in the area of anesthesiology. The zebrafish is an emerging animal model with many qualities that position it as an attractive alternative to perform basic biomedical research: it has high fecundity, has a short life cycle and the cost of infrastructure, supplies and reagents necessary for its upbringing and reproduction are low. The present work is a narrative review that shows studies that have used fish for drug research and anesthesia protocols. Likewise, the general characteristics of the zebrafish and some pioneering works of basic research in the field of anesthesiology are presented in which it has been used as an experimental animal. This information may be useful for professionals and researchers in the area of anesthesiology interested in performing basic biomedical research with the zebrafish as a study model.

Keywords: *Zebrafish; Anesthesiology; Basic Biomedical Research; Anesthetics; Animal Models*

Introduction

Preclinical research is fundamental in the field of health because it allows the identification of new substances with diagnostic and/or therapeutic potential, reevaluate known drugs and identify aspects related to the efficacy and safety of multiple substances and procedures. The results of preclinical trials can be extrapolated and implemented to the clinical field and form the basis of what is called translational medicine [1]. To carry out preclinical studies, various research models are used, including subcellular organelles such as mitochondria, vacuoles, vesicles, synaptosomes, biological membranes; cells and cell cultures; various isolated tissues and organs, up to complete living organisms and in this case, numerous animal models have been traditionally employed. In recent decades and thanks to the progress made in genetic and molecular biology techniques have been developed animal models, invertebrates and vertebrates, fairly refined that reproduce a diverse and large number of diseases that affect the human being: hypertension, obesity, diabetes mellitus, epilepsy, neurodegenerative diseases, arrhythmias among many others [2-6].

In the field of anesthesiology, basic research is not only aimed at the discovery and development of new substances, there is also interest in developing anesthetic induction protocols that can be adapted to the clinical veterinary or human field. Research related to anesthesia protocols seeks to establish the ideal sequence of steps and drugs that ensure an adequate transition between the state of consciousness and the desired anesthetic level: sedation, immobilization, narcosis, amnesia, in order to offer safety in invasive procedures that require the use of anesthetics and, additionally, that they allow euthanasia procedures in animals [7-9].

However, the regular use of common animal models such as rats, mice, guinea pigs, etc., have many limitations especially in economic terms, since in many occasions high-cost facilities, equipment and supplies are required. For this reason, there is a continuous search for low-cost vertebrate animal models and the zebrafish emerges as an economic animal model and very useful in basic biomedical research [10-12].

The present work is a narrative review that aims to show the general aspects of basic biomedical research in anesthesiology using fish in general and zebrafish in particular. The review shows that the use of zebrafish in basic research in the area of anesthesiology is limited, but it is a research tool with a wide potential.

Review of the literature

For the present review, a search was made of the existing literature from 2005 to 2017 in the databases of Medline, ScienceDirect, EMBASE and the academic Google search engine. The keywords for the search included fish, zebrafish, anesthetics, anesthesiology, which were combined with the "and" logic connector. Similarly, the terms rat and anesthesiology or anesthetics were used to compare the number of publications in which the rat model is used with the zebrafish model (Figure 1). Review articles and original articles closely related to the topic were selected and reviewed.

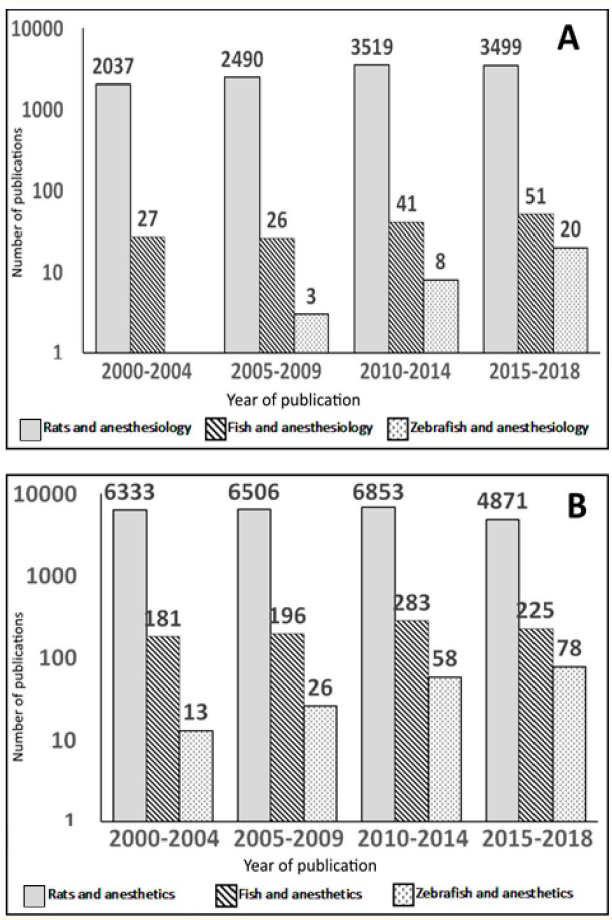

Figure 1: *Number of articles cited in the Pubmed database. A. Search results with the terms anesthesiology, rat, fish, zebrafish. It is observed that from 2005 publications appear in the field of anesthesiology using the zebrafish model. B. Search results with the terms anesthetics, rat, fish, zebrafish. It is observed how the rat model is the most used for efficacy and safety studies of anesthetics, the use of fish is lower and the zebrafish model appears in less than 40% of publications related to fish, however, its use has increased in recent years. Logarithmic scale in the axis number of publications.*

Anesthesia in Fish

Of all the articles cited in the databases, not all are related to the area of anesthesiology or to research related to conventional anesthetics. Of the publications that are directly related to the field of anesthesiology, the majority are aimed at evaluating drug efficacy and safety and drug administration protocols for anesthetizing fish in general. The number of publications related to research of anesthetics with the zebrafish as a model are more scarce. The use of anesthetic in fish is more common than you think, because in the fish area the production of commercial fish (rainbow trout, carp, salmon, tilapia) requires evaluation and regular clinical monitoring. Fish are often anesthetized to facilitate handling of specimens during blood sampling, biopsy collection or gamete collection for "in vitro" reproduction [8,13]. The topic of anesthesia and its characteristics is important in the area of fish research, because in many cases different types of procedures require the temporary immobilization of the fish, without this implying the risk of causing harm or death and the total animal recovery [7,8].

Evaluation of the Anesthetic State in Fish

For the evaluation of the anesthetic state of the fish the spontaneous movement that is reflected in the speed and direction of displacement in the water, as well as the coordination of the movements are evaluated. The reflex response to visual and tactile stimuli, balance, muscle tone and respiratory rate is also evaluated, which is evaluated by the movement of the operculum, fin that covers the gills organs that allow gas exchange in fish (Figure 2E). With these criteria have been described 4 levels ranging from normal consciousness, sedation, narcosis, anesthesia to the plane of medullary collapse, levels that can be reached more or less quickly depending on aspects such as type of anesthetic used, dose, via of administration among others (Table 1).

Level	State Clinical	Movement	Reflexes	Balance	Muscular Tone	Breathing
0	Normal	Active swim	Suitable	Normal	Normal	Normal
1.1	Mild sedation	Normal	Decreased response	Normal	Normal	Normal
1.2	Deep sedation	Decrease	Mild response	Mild alteration	Slight decrease	Slight decrease
2.1	Narcosis level	With position changes	Mild response	Loss of balance	Moderate decrease	It can increase
2.2	Deep narcosis	Absent	Vibratory stimuli	Absent	Moderate decrease	Moderate decrease
3.1	Mild anesthesia	Absent	Response to pain	Absent	Moderate decrease	Moderate decrease
3.2	Deep anesthesia	Absent	Analgesia	Absent	Moderate decrease	Moderate decrease
4	Spinal collapse	Absent	Absent	Absent	Absent	Decreased (Death)

Table 1: *Levels of anesthesia described in fish. The evaluation of the level of anesthesia is performed by evaluating parameters of movement, reflexes, balance, muscle tone and respiratory rate.*

Not only the induction of anesthesia in fish has been described in detail, also the recovery of the animal is key and for this reason scales have been developed that allow assessing the anesthesia status of the animal and performing an adequate follow-up [7,8]. The anesthetic recovery of the fish follows an inverse order to the induction of anesthesia, ranging from deep anesthesia to full recovery and three phases are described:

- State I: Immobility but movement opercular
- State II: uncoordinated body movements and opercular movement
- State III: Normal movement.

Types of anesthesia used in fish

The induction of anesthesia in fish can be done with physical means or with chemical means. The type of anesthetic chosen must ensure that the procedure and anesthesia do not interfere with the experiments and their choice will depend on aspects related to duration, depth of anesthetic state and toxicity of the selected anesthetic. In cases in which euthanasia of the animal is sought, toxicity is a sought-after factor that is avoided in normal procedures [7,8]. However, any anesthetic selected can be used for euthanasia procedures of the experimental animal.

Physical Means

The most used physical means are two: hypothermia and electric current.

Hypothermia

The most commonly used physical means to induce anesthesia in fish is hypothermia (cryoanesthesia) and there are works where it is evidenced how the immersion of the fish in water at 4°C reduces motor activity [14-17]. The anesthetic effect is moderate and includes decreased movement and decreased sensitivity. The mechanism of action is not clear, however, in some studies carried out in in vitro models, it is proposed that the effect of hypothermia would be related to GABA activity and modulation of the calcium signal in the nervous system, which allows to raise a possible GABAergic effect , an increase in inhibitory activity in various neural circuits that would explain the possible neuroprotective effect of hypothermia in some cases [16,17]. However, more studies are needed to confirm this theory. The method of hypothermia to induce anesthesia in fish has limitations, especially to perform studies related to the cardiovascular system because cold exposure has a direct impact on the regulation of the activity of this system.

Electrostimulation

It is a less used method and its use is more frequent in large species such as salmon and rainbow trout, among others, where it is easier to apply electrodes to perform the procedure. To induce anesthesia with electric current has used so much direct current, as alternating current. It has been reported that direct current (12V, 30 - 150 mA) produces anodotaxis (movement towards the negative pole of the source), electronarcosis and tetany; while the alternating current (110 - 220V) produces electronarcosis and tetany. It should be borne in mind that prolonged exposure can cause damage to internal organs and tissues [18,19].

Chemical means

They are the most used means and in general anesthetic drugs are used for veterinary and human use that can be classified as local anesthetics and general anesthetics, likewise hypnotic agents and vasoactive drugs used in resuscitation have been used (Table 2).

Anesthesia in Fish		
Physical Anesthetics Agents		
Type of anesthesia	**Dose**	**Species**
Hypothermia	Water with ice (4°C)	Salmon, tropical species
Electroanalgesia	AC 120 - 220V DC 12V; 30 - 150 mA	Various species
Chemical Anesthetics Agents		
Drug	**Dose**	**Species**
Tricaine (MS-222)	25 - 480 mg/L	Salmon, trout, carp, tilapia
Lidocaine	100 - 350 mg/L	Trout, zebrafish
Benzocaine	4 - 100 mg/L	Salmon, trout, carp, tilapia
Eugenol	6 - 150 mg/L	Salmon, trout, carp, tilapia
Halotane	0,5 - 20 ml/L	Carp
Isofluorane	0,25 - 0,75 ml/L	Carp, zebrafish
Phenoxyethanol	0,4 - 200 mg/L	Trout, salmon, carp, tilapia
Etomidate	1 - 7 mg/L	Salmon, trout, carp, tilapia
Quinaldine	15 - 70 mg/L	Salmon, trout, carp, tilapia
Ketamine	30 - 130 mg/Kg (IM)	Trout, salmon, zebrafish
Propofol	2,5 - 7,5 mcg/ml	Various species
Alfaxalone	12 mg/Kg (IM)	Salmon, trout
Carbon dioxide	200 - 1500 mg/L (1:1 CO_2 - O_2)	Salmon, carp
Analgesic Agents		
Buprenorphine	0,01 - 0,1 mg/Kg (IM)	Rainbow trout
Ketoprophen	2 mg/Kg (IM)	
Butorphanol	0,05 - 0,4 mg/Kg (IM)	Koi
Carprofen	1 - 5 mg/Kg (IM)	Rainbow trout
Lidocaine	0,1 - 2 mg/Kg (IM)	Trout, zebrafish
Anxiolytic agents		
Diazepam	1,5 – 5 mg/L	Various species
Fluoxetine	25 - 50 mcg/L	Various species
Buspirone	25 - 50 mg/L	Various species
Chlordiazepoxide	5 - 20 mg/L	Various species
Vasoactive Agents		
Doxapram	5 mg/Kg IV - IP	Various species
Epinephrine	0,2 - 0,5 ml IV – IP	Various species

Table 2: *Types of anesthesia used in fish. The different types of physical and chemical anesthetics used are observed, as well as some frequently used adjuvants. Included are reported doses and some of the commercial, ornamental or research species in which they are used.*

Methods of Administering Anesthetics in Fish

The administration of anesthetic drugs in fish can be done by two simple methods: topical or injectable methods.

Topical Methods

It is the most conventional and simple method because basically it consists of diluting the anesthetic in water; When the substance comes into contact with the fish, it enters the body through diffusion. The speed of absorption of the drug will depend on the characteristics of the drug in terms of lipid solubility, molecular weight, pKa, pH and temperature of the medium among others. This form of administration of anesthetics of chemical type is equivalent to methods of inhalation and topical administration in terrestrial species, or in humans, because the anesthetic comes in direct contact with skin, mucous membranes and gills. One of the advantages of this method is that the possibility of trauma is zero, but it must be ensured that the chemical characteristics of the anesthetic do not drastically modify the pH of the medium in which the fish is, as sudden changes in pH (extreme acidity or basicity) can compromise the life of the specimen [8,9].

Injectable Methods

Injectable anesthetics can be administered intramuscularly, intraperitoneally and even intravascularly. In this case there is a risk of visceral trauma, bruising and muscle damage [7,8,13].

Zebrafish as an Emerging Research Model

The zebrafish is a small freshwater fish, belongs to the cyprinidae family, is native to rivers in India and is distributed in regions of Bangladesh, Nepal, Nyanmar and Pakistan; however, it is common to find it in aquariums around the world because it has become popular as a pet. This implies that its acquisition is relatively easy and because it has a high capacity for reproduction, the risk of extinction is low, therefore, it is not a protected species. Multiple characteristics have contributed to the popularity of the zebrafish as a model in basic biomedical research [20]. One of the characteristics that makes this model attractive for research is that embryonic and larval development occurs externally and the process of development from fertilization to young adult age is rapid, since it is completed in about three months (Figure 2A-2D). An additional advantage is that, in the early stages of life, embryonic and larval, the zebrafish is transparent (Figure 2B-2D), which allows direct monitoring of the development and functioning of internal organs under normal conditions, with microscopy techniques conventional, without it being necessary to sacrifice the animal or place it in artificial microenvironments [10-13].

Reproduction and care of the zebrafish

The small size of the adult fish (3 cm X 2 cm X 1 cm, Figure 2E) guarantees the maintenance of numerous specimens in a small space which reduces the costs and space of the facilities, as well as the costs in the purchase and maintenance of equipment. The zebrafish is a species resistant to environmental changes, however, its use in research has forced to establish basic conditions for its reproduction and maintenance.

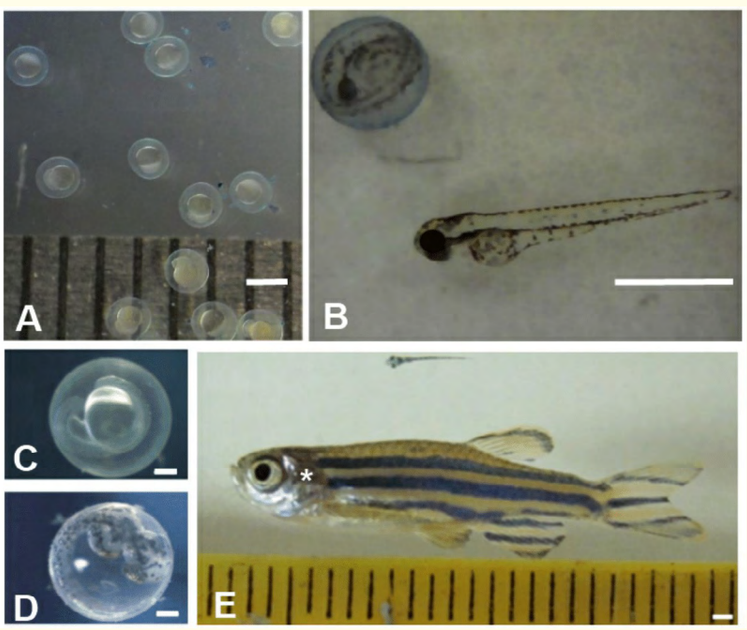

Figure 2: *Zebrafish model. A. Fertilized ovules. B. Embryo 24 hours post-fertilization (24 hpf, top left) and zebrafish larva (72 hpf, lower right). C. Embryo of 12 hpf; transparency is evident. D. Embryo of 48 hpf, we observe the transparency and the beginning of the typical pigmentation of this species. E. Adult zebrafish compared to the size of the larva at the top. In E the asterisk indicates the operculum that covers the gills. Scale in A, B and E 1 mm in C and D 0.2 mm.*

For the breeding and reproduction, aquariums are required, which can be of two types: static and recirculation. The static systems consist of individual aquariums with filtration system, aeration and own temperature control. The recirculation systems have a central unit that controls the temperature, pH and aeration of the water, which circulates continuously through connected tanks. Since the zebrafish is a highly sociable species it is recommended to keep 5 specimens per liter of water. The water must have specific conditions of pH (6.8 to 7.5), constant temperature (23 to 30°C) and must be exchanged around 10 - 25% weekly. The washing of aquariums and change of filters is done every month. Additionally, it must have a controlled lighting system that guarantees light-dark cycles (14 hours-10 hours). Regarding feeding, the zebrafish is omnivorous, but there are commercial preparations of granulated or flaked foods that guarantee a complete nutritional contribution; the food is administered once or twice daily and in the early stages of development it is recommended to administer live food as brine shrimp. The reproduction of the zebrafish is continuous, a female can produce around 200 ovules in each laying and the success of fertilization is high and about 90% of hatched ovules are fertilized. However, ova and embryos must be separated from adults, as the fish devour their young. In general, the players, females and males, must be separated and only placed in a single aquarium when reproduction is scheduled; they are usually placed in breeding aquaria in a ratio of 2 - 3 males per one female [21-23].

The cost of reproduction and maintenance of specimens and infrastructure is also low when compared to the costs of specialized habitat in rodents [24-26]. The need for animal handling is minimal, which reduces the requirement for technical personnel. It is also important to consider the ease of administration of drugs, especially water-soluble chemicals and the success of large-scale mutagenesis studies. On the other hand, there is ample documentation regarding bioethical standards that regulate its use in the laboratory [22,24,27]. There are also internet portals that contain banks of information related to genetic resources for study, databases and various protocols that include breeding methods and various research techniques. Currently there are several universities, including the University of Oregon, which offer the option of acquiring specimens or populations of wild zebrafish, as well as mutated and transgenic lines; similarly, various institutions offer a wide genetic information of the species [22,28-30].

In recent years there has been an increase in the number of publications in basic biomedical research that use zebrafish as a study model. The areas of research that increasingly use this model are numerous and include the areas of genetics [20,31], pharmacology [32], toxicology [33,34], neurosciences [35,36], biochemistry [37], cardiology [38-40], cancerology [41-42], among others. Although the use of zebrafish as a research model has increased in research centers in the USA and Europe, in Latin America it is relatively unknown and only some research centers in Mexico, Chile, Argentina, Brazil, Uruguay and Colombia use this model [43]

Anesthesia in Zebrafish

The zebrafish has been widely used in biology studies of the development of the different organs and systems of the fish, which include nervous system, cardiovascular system, renal system, gastrointestinal system, reproductive system. But it was two decades ago, from the 90s, that are taken as a reference for the study of diseases and its use in basic biomedical research is intensified, because it was possible to perform large-scale mutations using a mutagenic chemical: nitrosourea. This, added to the complete identification of the genome, has allowed us to carry out mutation analysis and relate it to gene functions, information that can be extrapolated to humans [44,45]. Parallel to the use of the research fish, anesthetic techniques have been refined in order to guarantee adequate handling during the experiments and to reduce the possibility of interaction with the interventions that are evaluated to avoid distortions in the results [7]. Many of the agents and anesthetic techniques used in humans have been evaluated, in fish especially in zebrafish, and include the use of physical means such as hypothermia, as a wide variety of chemical agents such as general anesthetics, local anesthetics and anxiolytics [46] (Figure 3A-3D).

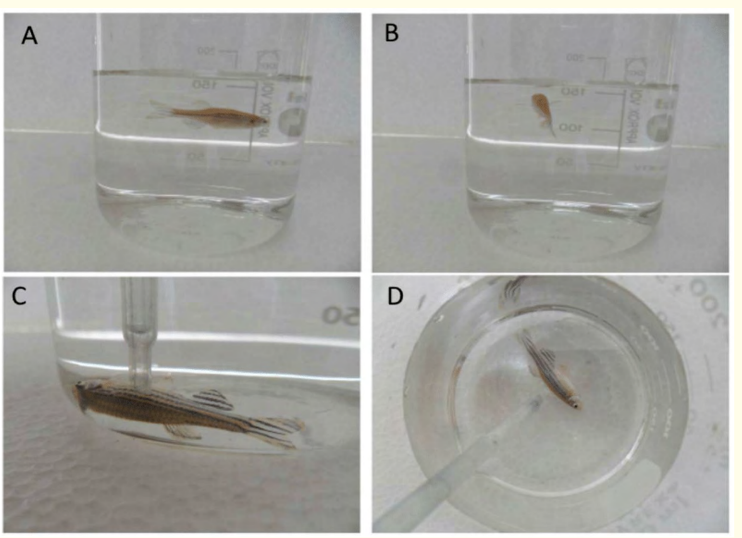

Figure 3: *Anesthesia in zebrafish with MS-222. A. Zebrafish side view, conscious state, adequate swimming pattern and normal balance. B Adult zebrafish rear view normal state. C. Zebrafish lateral position, state of deep anesthesia, the fish has no movement, there is loss of balance and there is no response to stimuli. D. Zebrafish seen above, state of deep anesthesia, without response to tactile stimulation.*

Hypothermia in Zebrafish

Hypothermia is one of the physical agents used in zebrafish for anesthesia and it has been reported that the combination of hypothermia with other anesthetics allows reducing the dose of drugs, which reduces their potential toxicity [14,15]. However, it must be taken into account that temperatures below 10 ˚C reduce the metabolism and activity of some organs and systems, which may interfere with results of potential experiments. It should be noted that the use of hypothermia in zebrafish is more oriented towards the development of optimal germ cell cryopreservation techniques [47-50].

Anesthetic agents in zebrafish

Tricaine of the anesthetic drugs, the most studied and widely used in fish is the tricaine known commercially as MS-222. In the case of zebrafish, tricaine has been used to induce sedation, anesthesia, and euthanasia. It is a substance that acts mainly blocking sodium channels, which affects not only the neuronal electrical activity, it also affects skeletal muscle and cardiac muscle, hence its potential cardiotoxic effect can be understood. It is usually used diluted in the medium at a concentration of 0.01 - 0.2 mg/ml at neutral pH, the time of onset of the sedative, anesthetic and recovery time are rapid, and it has a wide margin of safety [15,51,52].

Eugenol: Another of the anesthetics that is commonly used in fish and that has a wide margin of safety is eugenol, or clove oil, which has traditionally been used in dentistry as a lime and antiseptic anesthetic. A study conducted showed that eugenol induces anesthesia in zebrafish faster and with lower doses compared to tricaine, although the recovery times were longer. However, the authors recommend eugenol as an alternative anesthetic to tricaine, given the low dose required, the wide margin of safety, low mortality and low cost [53-55].

Other anesthetic agents. In another recent study Collymore and colleagues evaluated the effect of lidocaine, methomidate and isofluorane compared to hypothermia or MS-222 [56]. The sedative, anesthetic, recovery and mortality effect was evaluated for each of these agents. It was observed that the use of MS-222 and lidocaine at low doses (325 mg/L) are adequate to perform invasive procedures, however, lidocaine at high doses and isofluorane caused high mortality among adult fish, so the exclusive use of These agents is not recommended. Hypothermia and methomidate were useful to perform rapid procedures that do not cause pain. Similar results have been reported in the literature and it is also suggested that the combination of anesthetics, tricaine and isoflurane, decrease the dose required and with it the potential toxicity [52].

Ketamine is another analgesic and anesthetic agent that has been studied in zebrafish in subanesthetic doses, in order to evaluate the effect on behavior [57]. It is reported that doses of 20 - 40 mg/L reduce the manifestations of anxiety in the fish and reduce the levels of total body cortisol, however more studies are required to evaluate its effect at full anesthetic doses [57-59].

Anxiolytic Agents in Zebrafish

The effect of sedatives and hypnotics such as benzodiazepines has also been explored as the zebrafish emerges as a model for anxiety studies. It has been reported that the diazepam GABA-A receptor agonist produces anxiolytic effect at non-sedating doses, 1.25-5 mg/L; while chlordiazepoxide, another GABA-A agonist showed no anxiolytic effect at different doses. Buspirone, a serotonergic agent, has also shown anxiolytic effects at doses lower than those needed to produce anesthesia, less than 50 mg/L [60]. Similarly, it has been reported that fluoxetine, a serotonin reuptake inhibitor, also decreases anxiety in zebrafish at doses so low that they can be typical in wastewater where fluoxetine may be a further contaminant [61,62].

Other Pharmacological Agents

Of the drugs used in resuscitation, probably the most studied are agents that affect the autonomic nervous system, both cholinergic and catecholaminergic agents, because the zebrafish is an important model for studies of development, function and disorders of the cardiovascular system. Therefore, there is a large number of publications that show the effect of some of these substances on the development and function of the cardiovascular system [63-65].

In relation to studies related to muscle relaxants evaluated in the zebrafish, there are few studies where these substances are evaluated. Lin and colleagues used curare, some divalent cations and some aminoglycosides to evaluate the function of the zebrafish sensory system called the lateral line system [66]. The area of muscle relaxants is a field where the zebrafish model can be very useful to explore safety and efficacy of this group of agents. More recently, Xu and colleagues using the cardiac arrest model in zebrafish showed that substances such as midazolam and ketamine decrease neuronal apoptosis that occurs after cardiac arrest and that is related to the propagation of calcium waves in the cerebral cortex. The administration of ketamine increased the probability of survival of the zebrafish [67].

Administration of Anesthetics in Zebrafish

As in other species of fish, the drugs can be administered diluted in water when the drug is soluble so that the drug diffuses through the skin, gills or digestive tract, this method is ideal to administer anesthetics in embryo and larval stages. it implies minimal trauma; It is only necessary to guarantee that the drug does not modify osmolarity or pH, since sudden changes can compromise the life of the specimens under study. Injectable methods are widely used in adult zebrafish, it can also be used in larvae and embryos, however microneedles are required to ensure adequate volume with minimal trauma.

Conclusions

The zebrafish is an alternative animal model for basic biomedical research studies, given the high fecundity, the rapid development from embryonic stage to adult and the low cost of inputs and reagents necessary for its breeding and reproduction. Additional advantages of the zebrafish include the low cost in terms of infrastructure, time and money that it demands, compared to the use of other species. These may be important advantages to consider in developing countries, such as many of the countries in Asia, Africa and Latin America, where investment in science, research and development is low. Despite these advantages, the zebrafish has been little used in the area of basic research in anesthesiology, a field in which it can be used to evaluate drugs and propose anesthesia protocols that can be extrapolated to the clinical field.

Conflict of Interest

There is no conflict of interests.

Bibliography

1. Becú-Villalobos and Damasia. "Medicina traslacional, ¿moda o necesidad?" *Medicina (Buenos Aires)* 74.2 (2014): 170-172.

2. Srinivasan K and P Ramarao. "Animal models in type 2 diabetes research: an overview". I*ndian Journal of Medical Research* 125.3 (2007): 451-472.

3. West David B and Barbara York. "Dietary fat, genetic predisposition, and obesity: lessons from animal models". *The American Journal of Clinical Nutrition* 67.3 (1998): 505S-512S.

4. McKinney., *et al.* "Animal model of depression: I. Review of evidence: Implications for research". *Archives of General Psychiatry* 21.2 (1969): 240-248.

5. Smith Gwenn. "Animal models of Alzheimer's disease: experimental cholinergic denervation". *Brain Research Reviews* 13.2 (1988): 103-118.

6. Pearce AI., *et al*. "Animal models for implant biomaterial research in bone: a review". *European Cells and Materials Journal* 13.1 (2007): 1-10.

7. Neiffer Donald L., *et al*. "Fish sedation, anesthesia, analgesia, and euthanasia: considerations, methods, and types of drugs". *ILAR Journal* 50.4 (2009): 343-360.

8. Sneddon Lynne U. "Clinical anesthesia and analgesia in fish". Journal of Exotic Pet Medicine 21.1 (2012): 32-43.

9. Readman Gareth D., *et al*. "Do fish perceive anaesthetics as aversive?". *PLoS One* 8.9 (2013): e73773.

10. Bakkers Jeroen. "Zebrafish as a model to study cardiac development and human cardiac disease". *Cardiovascular Research* 91.2 (2011): 279-288.

11. Briggs Josephine P. "The zebrafish: a new model organism for integrative physiology". *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology* 282.1 (2002): R3-R9.

12. Hill Adrian J., *et al*. "Zebrafish as a model vertebrate for investigating chemical toxicity". *Toxicological Sciences* 86.1 (2005): 6-19.

13. Zahl Inger Hilde., *et al.* "Anaesthesia of farmed fish: implications for welfare". *Fish physiology and biochemistry* 38.1 (2012): 201-218.

14. Chen Kan, *et al*. "The evaluation of rapid cooling as an anesthetic method for the zebrafish". *Zebrafish* 11.1 (2014): 71-75.

15. Wilson Jolaine M., *et al*. "Evaluation of rapid cooling and tricaine methanesulfonate (MS222) as methods of euthanasia in zebrafish (Danio rerio)". *Journal of the American Association for Laboratory Animal Science* 48.6 (2009): 785-789.

16. Popovic Robert., *et al.* "Anesthetics and mild hypothermia similarly prevent hippocampal neuron death in an in vitro model of cerebral ischemia". *Anesthesiology: The Journal of the American Society of Anesthesiologists* 92.5 (2000): 1343-1349.

17. Feiner John R., *et al*. "Mild hypothermia, but not propofol, is neuroprotective in organotypic hippocampal cultures". *Anesthesia and Analgesia* 100.1 (2005): 215-225.

18. Barham WT., *et al*. "The use of electronarcosis as anaesthetic in the cichlid, Oreochromis mossambicus (Peters). I. General experimental procedures and the role of fish length on the narcotizing effects of electric currents". *The Onderstepoort Journal of Veterinary Research* 54.4 (1987): 617-622.

19. Barham WT., "Some observations on the narcotizing ability of electric currents on the common carp Cyprinus carpio". *Onderstepoort Journal of Veterinary Research* 56.3 (1989): 215-218.

20. Lieschke Graham J., *et al.* "Animal models of human disease: zebrafish swim into view". *Nature Reviews Genetics* 8.5 (2007): 353.

21. Ackermann Gabriele E., *et al.* "Zebrafish: a genetic model for vertebrate organogenesis and human disorders". *Frontiers in Bioscience* 8.1 (2003): d1227-d1253.

22. Koerber Amy S and Jennifer Kalishman. "Preparing for a semiannual IACUC inspection of a satellite zebrafish (Danio rerio) facility". *Journal of the American Association for Laboratory Animal Science* 48.1 (2009): 65-75.

23. Lawrence*., et al*. "Regulatory compliance and the zebrafish". *Zebrafish* 6.4 (2009): 453-456.

24. Markovich Michelle L., *et al*. "Diet affects spawning in zebrafish". *Zebrafish* 4.1 (2007): 69-74.

25. Kim Seongcheol., *et al*. "Modular, easy-to-assemble, low-cost zebrafish facility". *Zebrafish* 6.3 (2009): 269-274.

26. Lawrence Christian. "The husbandry of zebrafish (Danio rerio): a review". *Aquaculture* 269.1-4 (2007): 1-20.

27. Stephens Martin L and Nina S Mak. "History of the 3Rs in toxicity testing: from Russell and Burch to 21st century toxicology". Reducing, Refining and Replacing the Use of Animals in Toxicity Testing (2013).

28. Sprague Judy., *et al.* "The Zebrafish Information Network (ZFIN): a resource for genetic, genomic and developmental research". *Nucleic Acids Research* 29.1 (2001): 87-90.

29. Sprague Judy., *et al*. "The Zebrafish Information Network: the zebrafish model organism database". *Nucleic acids research* 34.1 (2006): D581-D585

30. Smith Stephen A. "Zebrafish resources on the internet". *ILAR journal* 53.2 (2012): 208-214.

31. Langenbacher Adam D., *et al*. "Mutation in sodium–calcium exchanger 1 (NCX1) causes cardiac fibrillation in zebrafish". *Proceedings of the National Academy of Sciences of the United States of America* 102.49 (2005): 17699-17704.

32. Rubinstein Amy L. "Zebrafish: from disease modeling to drug discovery". *Current Opinion in Drug Discovery* and *Development* 6.2 (2003): 218-223.

33. Rubinstein Amy L. "Zebrafish assays for drug toxicity screening". *Expert Opinion on Drug Metabolism and Toxicology* 2.2 (2006): 231-240.

34. Vargas Rafael and Johny Ponce-Canchihuaman. "Emerging various environmental threats to brain and overview of surveillance system with zebrafish model". *Toxicology Reports* 4 (2017): 467-473.

35. Souza Bruno Rezende and Vincent Tropepe. "The role of dopaminergic signalling during larval zebrafish brain development: a tool for investigating the developmental basis of neuropsychiatric disorders". *Reviews in the Neurosciences* 22.1 (2011): 107-119.

36. Vargas R., *et al*. "The zebrafish brain in research and teaching: a simple in vivo and in vitro model for the study of spontaneous neural activity". *Advances in Physiology Education* 35.2 (2011): 188-196.

37. Taylor Michael R., *et al*. "A zebrafish model for pyruvate dehydrogenase deficiency: rescue of neurological dysfunction and embryonic lethality using a ketogenic diet". *Proceedings of the National Academy of Sciences* of *the United States of America* 101.13 (2004): 4584-4589.

38. Nemtsas Petros., *et al*. "Adult zebrafish heart as a model for human heart? An electrophysiological study". *Journal of Molecular and Cellular Cardiology* 48.1 (2010): 161-171.

39. Vargas Rafael and Isabel Cristina Vasquez. "Cardiac and somatic parameters in zebrafish: tools for the evaluation of cardiovascular function". *Fish Physiology and Biochemistry* 42.2 (2016): 569-577.

40. Milan David J., *et al*. "In vivo recording of adult zebrafish electrocardiogram and assessment of drug-induced QT prolongation". *American Journal of Physiology-Heart and Circulatory Physiology* 291.1 (2006): H269-H273.

41. Amatruda James F., *et al*. "Zebrafish as a cancer model system". *Cancer Cell* 1.3 (2002): 229-231.

42. Moshal Karni S., *et al.* "Zebrafish model: worth considering in defining tumor angiogenesis". *Trends in Cardiovascular Medicine* 20.4 (2010): 114-119.

43. Allende Miguel L., *et al.* "First meeting of the Latin American zebrafish network". *Zebrafish* 8.1 (2011): 31-33.

44. Howe Kerstin, *et al*. "The zebrafish reference genome sequence and its relationship to the human genome". *Nature* 496.7446 (2013): 498.

45. Barbazuk W Bradley, *et al*. "The syntenic relationship of the zebrafish and human genomes". *Genome research* 10.9 (2000): 1351-1358.

46. Matthews Monte and ZoltAn M Varga. "Anesthesia and euthanasia in zebrafish". *ILAR Journal* 53.2 (2012): 192-204.

47. Guan M., *et al*. "Cryopreservation of zebrafish (Danio rerio) oocytes using improved controlled slow cooling protocols". *Cryobiology* 56.3 (2008): 204-208.

48. Higaki Shogo., *et al*. "Cryopreservation of primordial germ cells by rapid cooling of whole zebrafish (Danio rerio) embryos". *Journal of Reproduction and Development* 56.2 (2010): 212-218.

49. Bai Chenglian, *et al.* "Cooling rate optimization for zebrafish sperm cryopreservation using a cryomicroscope coupled with SYBR14/PI dual staining". *Cryobiology* 67.2 (2013): 117-123.

50. Yang Huiping., *et al*. "Development of a simplified and standardized protocol with potential for high-throughput for sperm cryopreservation in zebrafish Danio rerio". *Theriogenology* 68.2 (2007): 128-136.

51. Attili Seetharamaiah and Simon M Hughes. "Anaesthetic tricaine acts preferentially on neural voltage-gated sodium channels and fails to block directly evoked muscle contraction". *PloS One* 9.8 (2014): e103751.

52. Huang Wei-Chang *et al.* "Combined use of MS-222 (tricaine) and isoflurane extends anesthesia time and minimizes cardiac rhythm side effects in adult zebrafish". *Zebrafish* 7.3 (2010): 297-304.

53. Grush J., *et al*. "The efficacy of clove oil as an anesthetic for the zebrafish, Danio rerio (Hamilton)". *Zebrafish* 1.1 (2004): 46-53.

54. Macova Stanislava., *et al*. "Comparison of acute toxicity of 2-phenoxyethanol and clove oil to juvenile and embryonic stages of Danio rerio". *Neuroendocrinology Letters* 29.5 (2008): 680.

55. Sanchez-Vazquez Francisco J., *et al*. "Daily rhythms of toxicity and effectiveness of anesthetics (MS222 and eugenol) in zebrafish (Danio rerio)". *Chronobiology International* 28.2 (2011): 109-117.

56. Collymore, Chereen., *et al.* "Efficacy and safety of 5 anesthetics in adult zebrafish (Danio rerio)". *Journal of the American Association for Laboratory Animal Science* 53.2 (2014): 198-203.

57. Riehl Russell., *et al*. "Behavioral and physiological effects of acute ketamine exposure in adult zebrafish". *Neurotoxicology and Teratology* 33.6 (2011): 658-667.

58. Zakhary Sherry M., *et al.* "A behavioral and molecular analysis of ketamine in zebrafish". *Synapse* 65.2 (2011): 160-167.

59. Félix Luís M., *et al*. "Ketamine NMDA receptor-independent toxicity during zebrafish (Danio rerio) embryonic development". *Neurotoxicology and Teratology* 41 (2014): 27-34.

60. Bencan Zachary., *et al.* "Buspirone, chlordiazepoxide and diazepam effects in a zebrafish model of anxiety". *Pharmacology Biochemistry and Behavior* 94.1 (2009): 75-80.

61. de Abreu Murilo Sander, *et al.* "Diazepam and fluoxetine decrease the stress response in zebrafish". *PloS one* 9.7 (2014): e103232.

62. Airhart Mark J., *et al.* "Movement disorders and neurochemical changes in zebrafish larvae after bath exposure to fluoxetine (PROZAC)". *Neurotoxicology and Teratology* 29.6 (2007): 652-664.

63. Bakkers Jeroen. "Zebrafish as a model to study cardiac development and human cardiac disease". *Cardiovascular Research* 91.2 (2011): 279-288.

64. Brette Fabien, *et al.* "Characterization of isolated ventricular myocytes from adult zebrafish (Danio rerio)". *Biochemical and Biophysical Research Communications* 374.1 (2008): 143-146.

65. Hu Norman., *et al.* "Structure and function of the developing zebrafish heart". *The Anatomical Record* 260.2 (2000): 148-157.

66. Lin LY., *et al*. "Extracellular Ca(2+) and Mg(2+) modulate aminoglycoside blockade of mechanotransducer channel-mediated Ca(2+) entry in zebrafish hair cells: an in vivo study with the SIET". *American Journal of Physiology Cell Physiology* 305.10 (2013): C1060-1068.

67. Xu Dao-jie., *et al.* "General anesthetics protects against cardiac arrest-induced brain injury by inhibiting calcium wave propagation in zebrafish". *Molecular Brain* 10.1 (2017): 44.

Volume 4 Issue 6 June 2018