Review Article

Zebrafish Heart as a Model to Elucidate the Mechanisms of Sudden Cardiac Death

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ABSTRACT

The heart of zebrafish has been used as a simple, low-cost model to study development, structure and function of the heart from early to late stages of life. Also, it has been established as a model to study different cardiac pathologies generated through different methods. Cardiac pathologies include from functional disorders such as arrhythmia, to structural disorders such as hypertrophic heart disease; in many of them, genetic and molecular aspects have been associated. Noteworthy, some of these genetic and molecular factors have been invoked as causes of sudden cardiac death in humans. This adverse outcome is common in many cardiovascular pathologies and can occur at any age. Unfortunately, etiology and physiopathology of sudden cardiac death are unclear, so extensive research in this area is required. This mini-review highlight three related points: First, the relevance of sudden cardiac death in humans. Second, advances in knowledge of the development, function and pathologies in the zebrafish heart model. Finally, the possibility of using the zebrafish model for the study of sudden cardiac. For this review, a literature search was performed using the PubMed database and the search engine Google Scholar: the words sudden cardiac death, zebrafish, arrhythmias, cardiomyopathies were combined for this search.

Introduction

Sudden cardiac death is the most common lethal outcome of many cardiovascular diseases, and it can occur in any age group, race or sex. The etiology and pathophysiological mechanisms are unknown; however, an important genetic component has been suggested to be involved in the early stages of life, and cardiovascular degenerative changes are decisive in the late stages. This review shows the advantages of zebrafish model considering the genetic, molecular and physiological information available on cardiovascular development and function throughout its life cycle to study sudden cardiac death. Much of the genetic, molecular, physiological and pathophysiological evidence described in zebrafish coincides with the data collected about the possible causes and mechanisms of sudden cardiac death in other animal models and humans.
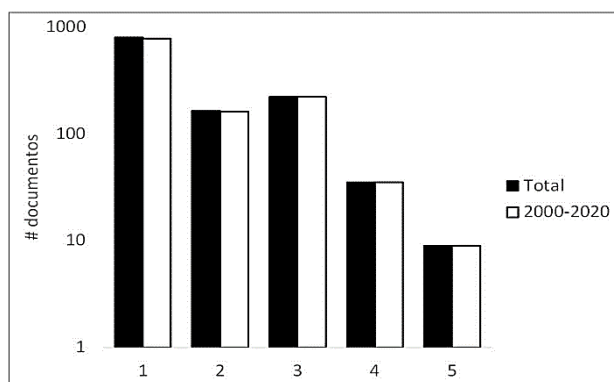
Literature Review

Extensive literature has been published related to sudden cardiac death, animal models, heart, zebrafish, arrythmias, cardiomyopathies and heart molecular biology (Figure 1). Yet, works contain little or no information on zebrafish as a model to study sudden cardiac death (Figure 2). For this review, the words sudden cardiac death, zebrafish, arrhythmias, cardiomyopathies, channelopathies and heart molecular biology were combined. The number of publications on the topic of sudden cardiac death has been increasing in the last decade (Figures 1 & 2). However, the number of works with zebrafish as a model to study sudden cardiac death is limited; so, this model and this topic have a lot of opportunities for researchers.

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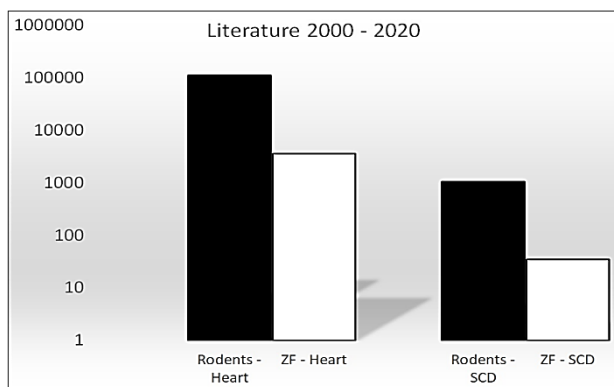
Figure 1: Results of literature review conducted from the PubMed database between the years 2000 and 2020. Search was performed with keywords "Rodents and heart", "zebrafish and heart", "rodents and sudden cardiac death" and "zebrafish and sudden cardiac death".

Figure 2: Results of literature review conducted from the PubMed database. Search was performed with keywords "zebrafish and heart molecular biology" (1); "zebrafish and cardiac arrhythmias" (2); "zebrafish and cardiomyopathies" (3); "zebrafish and sudden cardiac death" (4) and "zebrafish and channelopathies" (5). Black bars show total documents and white bars show documents from 2000 to 2020.

Cardiovascular Disease and Global Disease Burden

Cardiovascular diseases rank first as a cause of morbidity in humans worldwide, and sudden death is a common outcome of many of these diseases (coronary atherosclerosis, myocarditis, hypertrophic cardiomyopathy, valve disease, conduction system abnormalities) without discerning age, sex, race or geographical area [1-5]. Sudden death is defined as death that occurs unexpectedly within a 1-hour frame after symptoms start. The causes and mechanisms are unclear, but genetic, structural, functional and environmental factors, among others, are involved, and each will be decisive according to individual characteristics. For example, in children and young individuals, biological and genetic factors are considered determinants; at advanced ages, structural and functional degenerative changes are the main determinants [1-6]. These various possibilities compel the need for studies focusing on these factors; however, clinical research has multiple limitations, including high economic costs, complex logistics, and bioethical considerations, among others. Due to these disadvantages, basic research with animal models is a necessary alternative to investigate this area.

Cardiovascular Research and Animal Models

Multiple animal models have been used for preclinical studies and include invertebrates and vertebrates. The most widely used are *Drosophila melanogaster*, *Caenorhabditis elegans*, *Xenopus laevis*, rodents (rats, mice, rabbits) and birds [7]. Cardiovascular research conducted in these models include embryological and developmental studies, as well as morphological, functional, physiological, pharmacological and toxicological studies [8, 9]. Various published reports have revealed common genetic, molecular, structural and functional cardiovascular patterns for many of the studied species, suggesting the existence of preserved processes and signaling pathways throughout evolution [7]. These signaling pathways common to many species depend on specific expression of genes encoding well-defined molecules that regulate the development of particular cardiovascular structures (Table 1). Genes that are considered key in cardiovascular development and can also be regulated together include GATA, cmlc, nkx, Tbox, and grl, among others [10-12]. In recent decades zebrafish has become an ideal animal model in multiple areas of basic research due to the multiple advantages reported by various authors [7, 13-22]. These advantages include low cost for both acquisition and maintenance, high reproduction, external fertilization and embryogenesis, allowing earlier manipulation of specimens. The life cycle is short, and the period from fertilization to young adult age is completed in three months [13]. Finally, zebrafish has similarity in its genome to humans (approximately 70%, orthologous genes) [14].

Table 1: Common genes found in zebrafish and cause of sudden death syndrome in humans.

Zebrafish Genes	Protein	Function	Syndromes	References
SCN5A	Nav1.5	I_{Na}, AP, Ph0	BrS LQTS	Channelopathies [1, 2, 6, 38, 41, 72-78]
KCNK1-3-4	TWK-1/2; TASK-1; TRAAK	I_{KP}, AP, Ph0, Ph1, Ph2, Ph3, Ph4	SQTS LQTS	
KCNH2	HERG	I_{Kr}, AP, Ph3	BrS LQTS SQTS	
KCNJ2/12	Kir 2.1/2.2	I_{K1}, AP, Ph3, Ph4	LQTS SQTS	
KCNJ3/5	Kir 3.1/3.4	I_{KAch}, AP, Ph4	LQTS	
KCNQ1	KVLQT1	I_{Ks}, AP, Ph3	LQTS SQTS	
CACNA1C	Cav1.2	ICa,L, AP, Ph2	BrS	

RyR2	Ryanodine receptor	Calcium release	LQTS SQTS ARVD CPVT	Cardiomyopathy [74-76, 79-83]
Sarcomeric genes: cmlc1, amhc, myl7, vmhc, ttn, tnnt2	Troponin T, Titin. myosin light chain,	Contractility	HCM	

I_{Na}: Sodium current; I_{Kr}: the fast component of delayed rectifier potassium current; I_{Ks}: the slow component of delayed rectifier potassium current; I_{K1}: the inward rectifier potassium current; I_{KAch}: The acetylcholine-activated inward rectifier potassium current; I_{KP}: leak K+ currents; ICa,L: L-type calcium current; AP: Action potential; Ph0: Phase 0; Ph1: Phase 1; Ph2: Phase 2; Ph3: Phase 3; Ph4: Phase 4; BrS : Brugada syndrome; LQTS: Long QT syndrome; SQTS: Short QT syndrome; ARVD: arrhythmogenic right ventricular dysplasia; CPVT: catecholaminergic polymorphic ventricular tachycardia; HCM: hypertrophic cardiomyopathy.

Zebrafish in Cardiovascular Research

As a cardiovascular model, zebrafish has some significant advantages, particularly that the zebrafish heart develops rapidly and is functional at 2 days postfertilization (dpf) and that many of the genes that determine cardiac development have been identified [15, 16]. Additionally, normal and induced cardiovascular morphological and physiological changes can be observed directly using conventional microscopy techniques in zebrafish embryos and larvae due to their transparency [17-22]. Furthermore, during the first week of development, the larvae can survive without circulation because, unlike adult fish, oxygen consumption is independent of cardiac function, as tissues can meet their needs by the simple diffusion of oxygen from the medium [23, 24].

Cardiovascular Development in Zebrafish

The development of the cardiovascular system in zebrafish heart and blood vessels has its origin in the primitive mesoderm where differentiated cells produce a primitive heart disc. The disc progressively evolves into a cardiac cone, from which a tubular structure develops: the cardiac tube. Subsequently, and by specific segmentation and rotation processes, the cardiac tube produces the different well-defined heart chambers: a venous sinus, an atrium, a ventricle and the arterial bulb. These four chambers are arranged in series and are separated by narrowing heart valves that ensure unidirectional blood flow [10, 12, 15, 25-27]. At the same time, hemangioblasts differ from the mesoderm, which produces angioblasts and endothelial cells. At 24 hours postfertilization (hpf), a simple vascular circuit is formed comprising the dorsal aorta and axial vein [28]. From the aorta are derived the intersegmental vessels that will produce different vascular circuits [29].

Heart in Zebrafish: Intrinsic and Extrinsic Activity

Although the cardiac tissue of vertebrates has the properties of excitability, conductivity, contractility and automation, which allow it to work independently, cardiac activity is regulated by the autonomic nervous system (ANS), explaining the dense innervation of the vertebrate heart of vertebrates [30-34]. These characteristics originate in the heart of vertebrates and demonstrate dual cardiac activity-intrinsic activity and extrinsic activity-that is ANS dependent.

I Intrinsic Cardiac Activity

The intrinsic activity is typical of cardiac tissue cells, both noncontractile and contractile cells, which together will determine the electromechanical activity of the heart. Noncontractile cells determine pacemaker activity, while contractile cells determine cardiac mechanical activity. Both cell types present their resting potentials and action potentials, reflecting the electrical properties of the cell membrane. In zebrafish, the noncontractile cells present in the sinoatrial node generate pacemaker activity by spontaneous depolarization. Two hypotheses have been tested concerning the generation of this activity: the "membrane clock" hypothesis supported by the presence of cyclic nucleotide-dependent channels (HCN channels) which favours the ingress of sodium (if); the "calcium clock" hypothesis supported by coupling between intracellular calcium release through active ryanodine receptors and the electrogenic sodium-calcium exchanger (NCX) activation. Some studies have shown that both clocks are coupled in zebrafish heart [35, 36]. Similarly, the action potential of zebrafish cardiac myocytes shares the same characteristics as those in mammals, depends on the interaction of inward and outward currents such as sodium, potassium and calcium and has 5 phases (Table 1). Phase zero is characterized by a rapid ascent that depends on a sodium input current [37]. Phase 1 of repolarization depends on a potassium output current. Phase 2 comprises a plateau due to calcium ingress and potassium output. Phases 3 and 4 depend on potassium currents and cause the return to the resting potential (Table 1). Several studies have shown the presence of counterpart ion channels to those responsible for the intrinsic activity in the heart of mammals [38-41].

II Extrinsic Cardiac Activity

In the neural control of intrinsic cardiac activity, both the sympathetic nervous system (SNS) and parasympathetic nervous system (SNP) participate through two classic neurotransmitters: adrenaline and acetylcholine. Adrenaline works by stimulating cardiac activity through beta-adrenergic metabotropic receptors, while acetylcholine reduces activity by acting through M3 metabotropic receptors. However, some authors have argued that other neurotransmitters, such as GABA and some growth factors, could also be involved in regulating cardiac activity [42-46]. According to current evidence, in zebrafish, an autonomic control is established after the first week of development, implying that, during the first week, cardiac activity depends exclusively on the intrinsic activity. In mammals, asymmetry in cardiac autonomic innervation has been observed, resulting in functional differences. The sympathetic system, through the right stellate ganglion, innervates the sinus node and right atrium, while the left stellate ganglion mainly innervates the left ventricle. The effect of this asymmetry indicates that the activation of the right stellate ganglion increases the heart rate (positive chronotropism) by acting on the sinus node and generates an

anti-arrhythmogenic effect, while the activation of the left stellate ganglion stimulates the myocardium (positive inotropism) and tends to be pro-arrhythmogenic and hypertensive [47].

However, the parasympathetic system regulates cardiac activity through the vagus nerve, which originates in the brain stem in the neurons of the nucleus ambiguus and motor dorsal nucleus of the vagus nerve and its axons extend to intrinsic cardiac nodes located in the wall of the right atrium. Hence, the postganglionic neurons innervate the sinus node [48-50]. Due to this organization, the heart rate varies in time according to the balance between the sympathetic tone and parasympathetic tone. The relationship between alterations in cardiac innervation, particularly cholinergic-adrenergic innervation, and cases of sudden death in arrhythmias, acute myocardial infarction and heart failure has been reported, but the underlying mechanisms involved in these disorders remain unclear [47, 51]. These extrinsic and intrinsic properties present in fish makes zebrafish an ideal model to study intrinsic cardiac activity, neural activity control, its development and maturation and its participation in lethal cardiovascular disorders, such as sudden cardiac death, from the early stages of the life cycle [52-54].

Zebrafish and Heart Disease

The study of sudden cardiac death has been difficult because pathologies of different origins can trigger this phenomenon [4]. The causes of sudden death have ranged from alterations in ion channels and fulminant arrhythmias to metabolic disorders derived from ischaemic phenomena [1, 51]. However, the evidence has not clearly established the physiological mechanisms. In this sense, zebrafish can be an ideal model because models have been characterized to study specific cardiac pathologies. Zebrafish can be used to study the intrinsic properties exclusively in its first week of development, and the determinants of the connection between the heart and autonomic regulation can be evaluated in the second week or the alterations by senescence in the adult zebrafish [35, 39]. In the case of heart disease, models of dilated and hypertrophic heart disease have been established and the relationship between pathology and gene alterations (HAND, NkX2,5, T-box, EYA4 and cat) and/or specific proteins, troponin T, troponin C, titin and sarcomeric proteins [25, 55-61].

The zebrafish has also been a model for studies of the cardiac conduction system, and its alterations are reflected in arrhythmias [62]. Using mutagenic studies of ion channels, such as sodium channels, and optogenetic techniques, arrhythmias have been developed to understand the underlying physiopathological mechanisms [22, 63-66]. Finally, studies investigating cardiac regeneration and myocardial ischaemic disease have used the zebrafish model. Experimental protocols of hypoxia and reperfusion, surgical recession or cryoinjury of the myocardium in the zebrafish ventricle have simulated ischaemia and cardiac reperfusion, a common condition in humans that could also be related to cases of sudden death (Table 1) [67-71].

Conclusion

The wide acceptance of zebrafish in research, the increasingly abundant information on its normal and pathological cardiovascular processes, its short life cycle, its genetic and functional similarities to humans make it an integrated model option to study sudden cardiac death, a multicausal health problem affecting individuals of various ages, races and gender.

Conflicts of Interest

None.

Funding

None.

Consent

Not applicable.

Ethics Committee Clearance

Not Applicable.

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