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Review Article

Zebrafish as a promising animal model for study of spinal cord injuries

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Abstract: Spinal cord injuries (SCI) are a Gordian knot for concerned scientists. Many species of animals have been used to study their repair mechanisms. In the core research area, most experiments use rabbits, rats, mice, dogs, and pigs. These animals pose some problems, such as high costs and difficultly of postoperative care. Finding a promising animal model with the advantages of frequently-used animal models, while avoiding disadvantages, is important and urgently required to understand underlying neurodegenerative mechanisms of SCI. Zebrafish, due to certain characteristics, have attracted more and more attention. This review introduces the basic features and advantages of zebrafish, making it a promising animal model that will play a significant role in the study of SCI in future research.

Keywords: Zebrafish, animal model, spinal cord injuries

Introduction

Spinal cord injuries (SCI), resulting in axonal, neuronal, and glial cell injuries, are devastating to individuals [1]. Every year there are 12,000 new cases of SCI in the USA. The total number of Americans living with SCI is estimated at 276,00, with the global scenario far worse, approximately 500,000 suffering from SCI annually [2]. Curing this disease, while helping people recover limb function and rebuild confidence, is a key problem for clinical doctors and researchers. Many kinds of studies have been performed to explore the repair mechanisms of SCI. Robinson used stem cells, Bartlett used tissue engineering technology, and Li used drugs [3-5]. For safety and reasons concerning medical ethics, most research cannot be done in humans, only in animals. However, few studies have clearly expounded how to choose a suitable animal to study and why. The more suitable the animal selected, the better the results with high reference value for the disease.

Rats, rabbits, mice, dogs, and pig are frequently used animal models with common problems, including high costs, difficult postoperative care, and less reference value to human disease [6-9]. In recent years, scientists found zebrafish to have the benefits of frequently used animal models while avoiding their shortcomings, showing great potential in medical areas. Zebrafish are vertebrates with 87% genetic similarity to humans, suggesting that some experimental results from zebrafish may also apply to humans [10-12]. This review briefly describes the value and shortcomings of frequently used animal models and significantly expounds the zebrafish as an animal model with a significant role and bright future in the study of SCI.

Frequently used animal models

Rats, rabbits, mice, dogs, and pigs are frequently used animal models in medical experiments (**Figure 1**). Gao used rats to demonstrate that the combination of melatonin and Wnt-4 could promote neural cell differentiation into bovine amniotic epithelial cells with positive effects after SCI [13]. The present research team found that rats are cheaper and easily cared for after operations to repair SCI, though

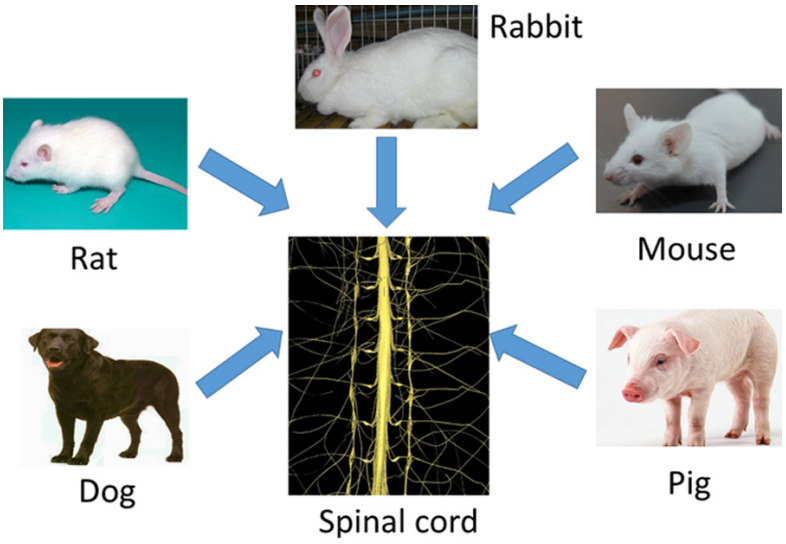

Figure 1. Frequently used animal models in SCI.

this result is in the experimental stage and far from clinical application [14]. Utada combined insulin-like growth factor-1 with erythropoietin to protect against ischemic spinal cord injury in rabbits, finding that Janus activated kinase 2 might contribute to protective effects [15]. Fandel transplanted human stem cells to mitigate mouse bladder dysfunction and central neuropathic pain after SCI [16]. Kim used dogs for experiments, suggesting that early intravenous injection of adipose-derived mesenchymal stem cells after acute SCI may prevent further damage through enhancement of anti-oxidative and anti-inflammatory mechanisms [17]. Cho used Chitosan in pigs to provide a novel medical approach of reducing the catastrophic loss of normal behavior after acute spinal cord and brain injuries [18]. A rough summary of previous research shows that rats have been widely used, followed by rabbits, mice, dogs, and pigs. Monkeys and apes have been used in a few experiments [19, 20]. Each animal model has merits and shortcomings. Monkeys and apes have a similar anatomy and physiological function of the spinal cord to humans, suggesting that experimental results have higher reference value to clinical treatment. Shortcomings have higher costs and difficult management after operations. Quadrupedal pigs and dogs give experimental results with less meaning than higher animals, but the costs are relatively low. Lower animals, such as rabbits, mice, and rats, have the advantages of being individually small, easily managed, low costs, with strong anti-infection ability and vitality. They could be useful for large-scale experiments, with imponderable advantages, compared to other animal models, despite their spinal cord anatomy and function being far from human [21].

For observing paraplegia limb functional recovery, apes and monkeys are the best model since they can stand. Pigs and dogs, which stand and walk on four limbs, are next [22]. Regarding cost, rats, mice, rabbits, and dogs are better than higher animals due to their cheap price and widespread use [23]. For experimental purposes, studies on the reconstruction of the micturition reflex arc after SCI use dogs and rats. Establishment of a central pain model usually selects rats, while spinal firearm wound models often use pigs [24-26]. Overall, low-level animals are chosen in the primary stage of experiments and higher animals when experiments are closer to success.

Researchers have been investigating whether there is an animal model with the merits of frequently used animal models, which have similar anatomy and physiological function of the spinal cord to humans, but also the merits of being individually small, easily managed, and low costs. These advantages make results more valuable to spinal cord studies in humans.

Zebrafish

Effectively establishment of animal disease models are conducive to further study of diseases, especially in SCI. Compared with rats, rabbits, dogs, and other classic model organisms, the zebrafish is a vertebrate with the merits of small size, being transparent to allow observation of organ development *in vitro*, having high fecundity, and a short generation time [27, 28]. Additionally, they reveal high similarity of characteristics with humans in their genome, proteome, and pathogenesis, making them an ideal organism for researchers of embryonic development and physiology, leading to their

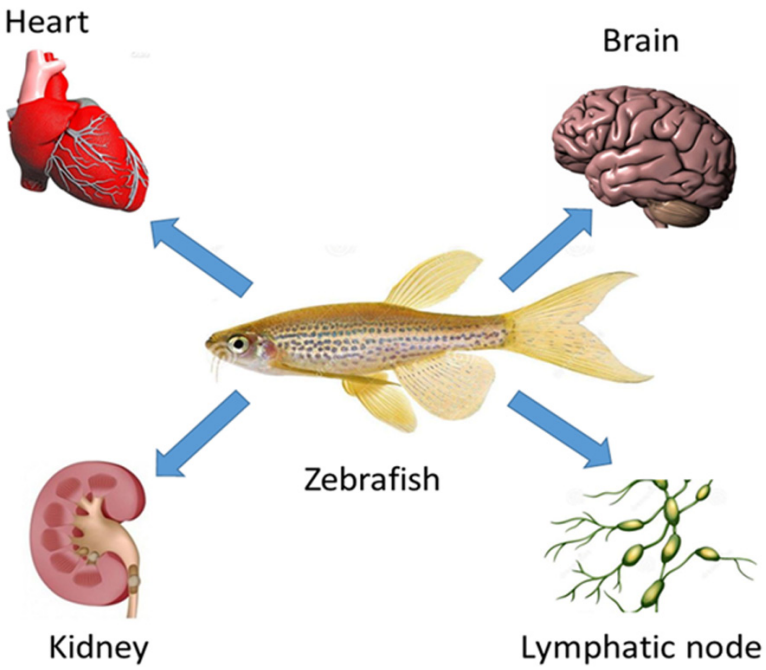

Figure 2. Zebrafish used in biomedical research.

widespread use in biomedical areas [29-31] (**Figure 2**). Many experiments have used zebrafish to do research, obtaining some promising results. Bournele used zebrafish to research cardiovascular disease, as its heart can regenerate throughout its lifetime, providing novel insight to understand human cardiac regeneration [32]. Sumio Isogai used zebrafish to elucidate the nature of the lymphatic system and provide a firm morphological foundation for molecular genetic research of lymph angiogenesis [33]. Sander outlined the current knowledge on zebrafish kidney formation, describing methods for inducing acute injury and focusing on the unique capacity of zebrafish adult kidneys to undergo de novo nephron formation when damage occurrs [34]. Lim demonstrated that Spred-2 signaling is important for cell proliferation, neuronal differentiation, plasticity, and survival in the cell proliferative phase during neural repair in injured zebrafish brains [35].

Zebrafish, used in SCI, have revealed many merits, such as repair of severed axons, replenishing lost cells, and inducing neurogenesis after injury, thereby regaining functional loss [36, 37]. Through analysis of pathophysiological processes in zebrafish after SCI, this was found to be quite different from frequently used animal models (**Table 1**). The pathophysiology in zebrafish is as follows [38, 39]: ① Present a brief inflammatory response to SCI and provide a positive environment for neurogenesis; ② Two types of macrophages appear in the lesion which provide an environment to promote axon regrowth: proinflammatory macrophages (M1) and anti-inflammatory macrophages (M2), especially the latter; ③ Increase expression of anti-apoptosis factor Bcl-2 and Akt, leading to minimal cell loss and motor neuron survival; and ④ Hyperplasia of glial cells and formation of glial scars are key factors that inhibit axon regeneration after SCI in mammals, but this situation could not be observed in zebrafish which generate a permissive environment for axonal regrowth. FGF has been considered a key factor regulating glial cell proliferation, migration, and morphological changes that lead to the formation of glial bridges on spinal cord injury sides and allows regeneration of axons passing through the lesion. Thus, glial cells play different roles in different central nervous systems.

Sasagawa suggested that activation of e2f4 after SCI may be responsible, at least in part, for significant recovery in zebrafish. Lin demonstrated that contactin-2 contributes to locomotor recovery and successful regrowth of axons after SCI in adult zebrafish. Yu reported that miR-133b suppresses the molecules which inhibit axon regrowth to promote the capacity of adult zebrafish locomotor function to recover after SCI [40-42]. Using zebrafish to study the development of the nervous system and to establish a model of SCI will provide a new theoretical basis to further explore the repair mechanisms of nerve injuries. Analysis shows that zebrafish are a promising animal model in SCI, as follows. Zebrafish are cheaper, they quickly proliferate, and are easy to maintain.

Zebrafish are a tropical freshwater fish, often raised in people's homes for enjoyment. Co-

Table 1. Comparison of pathophysiological process in zebrafish and frequently used animal models after SCI

Zebrafish	Frequently used animal models
Present a brief inflammatory response	Cause severe inflammation
Macrophages involved in clearing myelin debris appear in the wound site	Proinflammatory macrophages appear in the wound site
Upregulate anti-inflammatory M2 type macrophages related molecules	Cell death cause huge neuronal and glia loss demyelination
Very minimal cell loss due to necrosis and apoptosis after injury	Release of toxic myelin breakdown product axonal degeneration
Proliferative response and extensive neurogenesis	Formation of fibroastrocytic scar
Generate permissive environment for axonal regrowth	Loss of astrocytes and oligodendrocytes and a much higher proportion of neurons

mpared to other animals, they have many advantages when used in SCI [43-45]: ① Pigs, monkeys, and dogs are ideal animal models to research SCI. However, a big body is a problem, as you must find a large lab to raise them which is inconvenient. An adult zebrafish's size is just 3 cm to 4 cm. It needs a small place with much convenience to the researcher; ② Larvae can feed 120 hours after fertilization and live for five days without feeding by consumption of the yolk. This gives zebrafish the benefit of avoiding the breeding problems of monkeys, apes, and rats, which require heavy daily work from laboratory staff. In addition, zebrafish living conditions are a pH from 6.5 to 7.5 and water temperature from 25°C to 31°C, easily achieved and maintained; ③ SCI research needs a large number of animals which usually do not come from a common ancestor. For example, one rat could not produce as many offspring at one point. One would need to wait a long time for the same source, which is important for research, deciding whether results are meaningful. Zebrafish generation intervals are short, usually three months. They could produce large numbers of eggs per brood, usually 100-200 eggs per clutch, to provide enough animal models to perform all analyses at each data point, ensuring the animals come from the same zebrafish; ④ SCI experiments have a certain mortality rate after surgery often caused by urinary system infections, especially in the complete spinal cord transected injury model. Therefore, one needs to increase the number of animals to prevent death when designing the experimental phase, leading to high costs. High reproducibility and quick development make zebrafish cheaper; ⑤ Embryos are transparent and observable from early larval stages, which allows a readout of morphogenetic processes that occur earlier in development. Furthermore, organs and tissue can be visualized *in vivo* and examined instantly. This merits not only the study of repairing mechanisms of SCI, but also investigating the developing spinal cord from embryo to adult. Fully understanding spinal cord development processes is the basis of repairing SCI.

Zebrafish gene functions have similarity with humans

The purpose of animal experiments is to apply results to humans. However, species differences hinder the progress of research. Monkeys and apes are closer models, but fees are high and they are difficult to care for. Rats and rabbits are cheaper, but the results have less value than monkeys and apes. In a previous study, Howe reported that the zebrafish genome contains approximately 26,000 genes, while 70% of human genes have at least one unambiguous zebrafish ortholog. Varshney compared 3,176 potential human disease genes listed in Online Mendelian Inheritance in Man (OMIM) database to the list of zebrafish genes, showing that there are 2,601 (82%) present in zebrafish [46, 47]. These results have an important consequence. Zebrafish gene similarity with humans gives potential for studying vertebrate development, biological pathways, and human disease. Precisely speaking, using zebrafish as an animal model for experiments has large value in researching human disease. For example, Bakkers and Santoriello found that zebrafish genes are remarkably similar to humans, making an excellent vertebrate model suitable for human congenital anomaly functional studies [48, 49]. Recent research in zebrafish has revealed several new intrinsic factors involved in successful axonal regeneration, such as cysteine and glycine-rich protein 1a, contaction-2, major vault protein, and miR-133b, whose functions in mammalian neurons require further investigation [50].

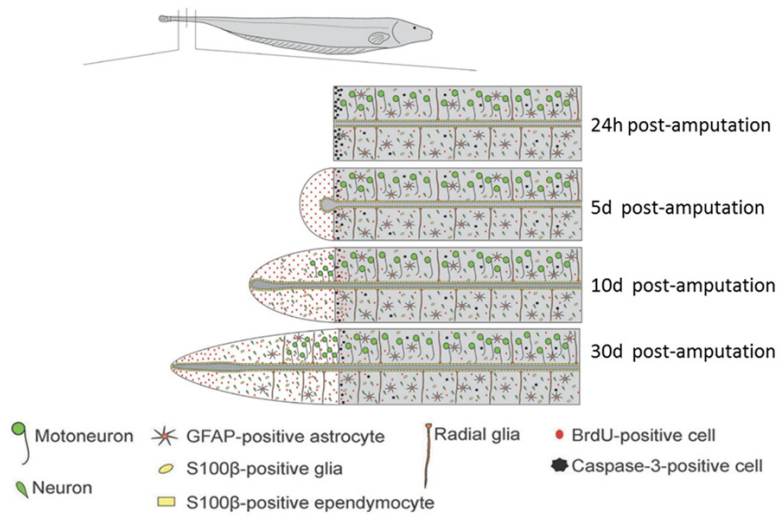

Figure 3. Overview of some of the major processes involved in spinal cord regeneration of teleost fish (picture from Zupanc, G.K and Sirbulescu, R.F.'s manuscript that was published in European journal of Neuroscience, 2011, 34(6): 917-29.

Sequencing of the zebrafish genome has provided several molecular tools (microarray, next-generation sequencing, transgenic animals, and morpholions) to perform functional studies that can verify the level of involvement of certain molecules and pathways in axonal regeneration [51, 52]. Fully understanding the relationship between zebrafish and human genomes will help identify roles for human genes from zebrafish mutations and help identify zebrafish models for genes identified by human disease. In the early stage of experiments, zebrafish could be used as an animal model to research genes that have a positive or negative role in SCI. If results are promising, the study could be used in higher animals for future research and application in humans. Zebrafish gene functions are similar to humans, suggesting that study results are more meaningful and have a higher reference value than with rats and dogs.

Successful and failed axonal regeneration existing in spinal cords

Zebrafish have a high-level ability for neurogenesis and synapse formation as well as maintaining low levels of apoptosis in response to nerve injuries. These make the zebrafish a promising animal model for study spinal cord regeneration (**Figure 3**) [53]. Brainstem neurons are able to regenerate axons across SCI area and extend caudally. This process requires approximately 4-6 weeks after complete spinal cord transection, typically accompanied by anatomical restoration at the lesion site and impressive functional (swimming) recovery [54]. However, not all axons could regenerate in the zebrafish spinal cord after injury. Becker's group found that 32%-51% of neural cells in the nucleus of the medial longitudinal fascicle, magnocellular octaval nuclei, and intermediate reticular nuclei could regenerate axons across the lesion and up to 4 mm into the caudal stump. However, neurons in the red nucleus, dorsal root ganglion neurons, and mauthner neurons failed to regenerate axons [55]. This result suggests that zebrafish can be used as an animal model to research mechanisms of successful and failed axonal regeneration after SCI in the same fish when the extrinsic factors are constant, avoiding differences encountered between two animals. This benefit will give results a higher reference value.

Research on molecular and signal pathway synergistic effects

From Zeynab's study, it is known that zebrafish have two types of pathology after SCI: acute response phase (less than 24 hours) and chronic phase (more than 1 day) [56]. Different molecular and signal pathways have different functions that have been researched in the acute and chronic phase after SCI: ① Wnt/β-catenin signaling, L1.1, L1.2. The major vault protein (MVP), contacion-2, and high mobility group box 1 (HMGB1) had positive effects on axonal re-growth while Ptena has an inhibitory effect; ② Neurogenesis is stimulated by Wnt/β-catenin signaling as well as HMGB1, but inhibited by Notch signaling; ③ Glial cells proliferate in response to fgf signaling, which causes glia bridge formation in favor of axonal regeneration; ④ In the acute phase, HMGB1 stimulate inflammatory response around the injury and suppress regeneration, as well as LPA,

which also induces microglia activation and neuronal death in addition to glia cell proliferation, but prevents neurite sprouting. The relationship between these functions and whether they have synergistic effects is not clear and needs to be explored.

Conclusion

Many types of animals are used in SCI. Rats are the most common, followed by rabbits, rats, mice, dogs, monkeys, and apes. Disadvantages of these animal models inhibit their widespread use. The zebrafish is a promising animal model used in many biomedical areas, showing positive results. In the repair of SCI, it has the advantages of being cheaper, quick to proliferate, and easily maintained. Zebrafish could be easily applied in experiments. Gene functions are similar to humans, giving results that are more meaningful and of higher reference value than other models. They have a high-level ability for neurogenesis and synapse formation, allowing in-depth research of repair mechanisms of SCI. Zebrafish spinal cords have the merit of axonal success and failed regeneration at the same time, thus they can evade the differences between two animals when studying regeneration. In conclusion, the zebrafish is a promising animal model in targeting functional regeneration of the spinal cord in humans.

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