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The use of zebrafish to evaluate neuropharmacology of the gold nanoparticles

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REVIEW ARTICLE

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ABSTRACT

Zebrafish (*Danio rerio*) is a vertebrate animal used in animal model research with complex brains and behaviors similar to humans and associate with low coast become a model attractive for the academic community to seek zebrafish for scientific research. Studies on diseases of the central nervous system (CNS) have advanced and news therapeutic agents were developed for treatment these disorders. Reports suggest that the zebrafish model supports the neurodegenerative studies due functional conservation between human genes implicated in neurodegenerative disorders. The discovery of therapeutic compounds for CNS using the zebrafish model allows to show a neuroprotective action or neurotoxicity that might alter the behavioral changes. Neurotoxicity tests might perform in zebrafish's embryos into 96 multi-well plates, which reduces the amount of substances used and cost. The bioactive compounds able to penetrate the blood-brain barrier (BBB) have important role physicochemical properties that might be desirable pharmacological effects and zebrafish trials allow if the substances might penetrate BBB and to exert central activity. The assays zebrafish are used to analyze nanoparticles that are small molecules used to explore variety applications in human health. Gold nanoparticles (AuNPs) has important properties which are extremely interest for pharmaceutical area such as drug delivery, cellular imaging, diagnostics, and therapeutic agents. Gold nanoparticles enhances Parkinson symptoms and improved neuroinflammation. Some studies show zebrafish might use to evaluate gold nanoparticles for human health hazard and toxicity studies. There is enormous potential for zebrafish in preclinical assays due to predict pharmacological and toxicity effects. Specific guidelines focused on methodologies in the zebrafish are needed to ensure adequate reproducible trials.

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1. Introduction

Nanotechnology is increasing around the world and being used in a variety of industrial sectors, including tissue engineering, drug delivery, imaging, diagnostics, surface texturing, and bio-interfaces which are currently using nanomaterials in their products. In 2020, there was a projection of approximately 7.6 billion in nanotechnology market industry USD [1,2]. Nanotechnology is defined as the designing, development, description, and applications of materials at nanometer scale, as well as the possibility of versatile rearrangements in their size, shape, chemical proprieties, and surface. It can also offer new solutions to the development of nanodrugs that present improved efficacy and safety [3-6].

In pharmaceutical field, for example, surface nanomaterials have important role in drug delivery. The biocompatibility of nanomaterials can be changed and their cell specific targeting ability and improve by attaching them with targeting ligand [4,7,8]. The demand of nanomaterial in medicine have been growing since last decade along with the area of nanotoxicity, which has raised considerably during the last 10-15 years, and the perspective is to continue in development [9,10]. Some toxicological studies of nanomaterial are conducted using the zebrafish model [11,12].

Native to Southeast Asia, Zebrafish (*Danio rerio*) has been accepted by the scientific community as an animal model which use is progressively increasing in the fields of toxicology and biomedical research [13-17]. The adult fish and larva have the availability of comprehensive behavioral catalogs which enhance the utility of this model species for translational neuroscience animal experimental model [18].

Zebrafish model presents some advantages such as low-cost, small size animals that can be easily handled, great reproducibility rates, and quick development. The transparency of the embryo provides the possibility to observe all cells since early larval stages, useful in chemical screen tests and acceptation in genetic tests [19,20]. In addition, the digestive, nervous and cardiovascular systems of this model animal are similar to mammal ones [15,21,22]. There are highly conserved signaling pathways between zebrafish and humans with a considerable degree of morphological, physiological, and genetic homology to humans [1,23,24].

Amongst several nanomaterials, gold nanoparticle has optical and surface plasmon resonance proprieties that allow it

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to be the first choice for research in pharmaceutical and biological fields. This optical propriety might be used in ultrasensitive detection and imaging-based therapeutic techniques requested for the treatment of diseases. Besides, gold nanoparticle is the subject of a preclinical trial underway for medicinal applications in therapy, diagnostics, and drug delivery vehicles through conjugation with biological and biocompatible ligands (drug vectorization and DNA/gene delivery) [25-28].

In this review, we highlight both larval and adult zebrafish extensively used in central nervous system research and targeting various brain disorders. We emphasize some pharmacological and toxicological methodologies that are capable to recognize the potential of zebrafish for translational neuroscience. Besides, we discuss the growing role of gold nanoparticles in biomedical research, and the use of zebrafish to evaluate pharmacology and toxicology.

2. Importance of zebrafish model in evaluations in Central Nervous System (CNS) studies

More than millions of the people suffer from neurological disorders that have a large social and economic impact around the world. Neurodegenerative diseases are a classic example of neurological disorders, such as: Alzheimer's disease (AD) Parkinson's disease (PD), Huntington's disease and amyotrophic lateral sclerosis (ALS). In general, these neurological disorders have common features in regional cytosolic or nuclear protein aggregation that it can lead to degeneration and death of particular neuron types. Consequently, there is a rapid loss of brain processes: cognitive and/or motor neuron function, for example [29,30].

Recent advances in neurology area show a great promise for the future utility in studying such diseases. The zebrafish model has been widely applied to study the mechanisms and pathogenesis of neurological disorders and diseases linked to the central nervous system. It supports an extensive array of experimental tools and techniques assembled to explore the knowledge associated with several neurodegenerative disorders [31,32]. Zebrafish has a central nervous system similarly organized to that of other vertebrates and with optimal described at multiple life stages [33]. Furthermore, there is a degree of functional conservation between human genes implicated in neurodegenerative diseases and zebrafish orthologues, which enables identification of molecular drug targets [34-37]. Zebrafish model of recessive parkinsonism suggests that Parkin or Pink1 knockdown gave rise to specific loss of dopamine neurons. Another issue observed is the zebrafish model of recessive spinal muscular atrophy: loss of Smn1 function caused specific motor axonal defects. Pathological features in several diseases were reported through transgenic overexpression of some protein mutants present in humans, e.g., Tau, Huntingtin, and SOD1. In certain cases, the cellular pathways of some specific diseases with posttranslational changes of some proteins were found in zebrafish model with the same abnormalities found in human diseases. The analysis of phylogenetic conservation of interacting proteins and relevant biomolecular pathways is adequate to ensure appropriate cellular handling of human proteins in zebrafish brain, in a manner that reflects current understanding of pathogenesis. The findings suggest that zebrafish can become a safety setting in to model the molecular underlying human neuropsychiatric disease [36,38-43].

Pain is a condition caused by damage to or dysfunction of the central nervous system, and it is frequently a component of many neurological disorders [44] Zebrafish can also be utilized in different models of pain studying, because it has conserved pain receptors and it gives the essential role of the opioid system in pain control, and it becomes an excellent pain translational model to pain research. The zebrafish opioid system might be a fundamental tool to understand the pain pathophysiology, and both adult and larvae zebrafish response to nociception are similar to mammals' ones [45-47]. The zebrafish behavioral changes might be shown through the hypoactivity caused by nociceptive substance, which can be easily assessed using automated video-tracking recordings. For example, 0.1% acetic acid impairs the zebrafish's locomotion, while aspirin (2.5 mg/L), morphine (48 mg/L) and lidocaine (5 mg/L) prevent this effect. The behavioral manifestation of nociception has negatively impacted overall mobility, resulting in the reduction of the total ambulation's zebrafish, while the administration of antinociceptive substance improved the nociception behavior [48-50].

Zebrafish has contributed to neurobehavioral studies through evaluation of drug action in central nervous system or examination of behavioral activities such as anxiety, sleep, addiction, social interaction, olfactory-related behaviors, effects of drug abuse, learning, and memory [51-60]. Adult zebrafish might be used to check anxiety-like effects of new pharmaceuticals in preclinical screening. Anxiety is evoked when the animal is exposed to experimental stressor situations and/or pharmacological agents. There are some methods used to evaluate anxiety-related phenotypes in zebrafish, and the most common models are light-dark test (LDT) and novel tank test (NTT) [33,56,58]. NTT is an experimental method similar to the open field one. The methodology is used to assess anxiolytic activity in zebrafish that is exposed to the experimental challenge in a pretreatment beaker before being transferred into the novel tank. In the new environment, the animal seek protection, and it remains in the bottom until it feels safe enough to explore. Researchers are able to analyze and compare anxiety through assessments such as latency to emerge to the upper half; transitions to the upper half of the tank; erratic ambulation, and freezing bouts. Normally, anxiety-related phenotypes in zebrafish indicated a longer latency to enter the upper half, time spent in the top reduced, erratic movement and freezing. Video tracking improves the research since multiple zebrafish might be recorded, analyzed, and, if necessary, re-analyzed. LTD method is based on the zebrafish has an innate preference for dark rather than light areas. This behavior is similar with the natural tendency of wild zebrafish, which does not prefer a light environment in order that not be detected by potential predators [56,61-65]. The zebrafish model is important to verify the therapeutic agents with central activity; however, it is required to investigate a possible neurotoxicity that it might provoke behavioral changes.

3. The use of zebrafish to toxicological evaluations

The discovery of potential therapies for neurological disorders is a great challenge that it is involving an optimization of animal model for drug screening [29,30,66-68]. In pharmaceutical drug discovery, the number of substances discovered in early target - or phenotypic-based screens for drug candidates-overcome the number that advances as clinical candidates. However, many of these compounds show relatively few to none progress to clinical trials [69]. In addition, there are factors that might influence the types of development-limiting toxicities observed in a research, for instance, bioactive compounds targeting CNS will require particular physicochemical properties that are more frequently associated with some undesirable effects such as pharmacological promiscuity [69].

Zebrafish allows advances in drug screening and it will likely add massive amount of data to behavioral pharmacology field. Some compounds can present a neuroprotective action which has been proved by using the animal model. Moreover, drug delivery of substances to the brain has an important role in the discovery and development of new central nervous system disease treatments [70]. The most important challenge

is the efficacy and safety, which it should address with an issue in their security and that there is no biotoxicity during drug design and development [69,71]. Zebrafish model is a vertebral animal model with complex brains and behaviors similar to humans that allows statistical reliability, feasibility for modification, and more stringent regulation. This model system enables research in neuropharmacology and neurotoxicity to combine complex behavioral phenotyping with high-throughput chemical screening. As behavioral datasets grow, researchers are applying new analytical approaches to explore, organize, and discover correlations between phenotypic patterns and compound treatments [70,72-74].

Neurotoxicity studies utilize zebrafish (adult and larvae) due to its small size: they can be easily handled, tests are generally performed by placing embryos in 96 multi-well plates which reduces the amount of waste and chemicals used, as well as lower costs. Chemical solutions and the compounds penetrate the embryo's membrane by passive diffusion. Zebrafish embryos are considered ideal for high-throughput screening and can be used to monitor the phenotypic and genotypic abnormalities upon exposure to bioactive compounds [14,75,76]. Literature suggests that the blood brain barrier (BBB) in zebrafish appear 3 days after fertilization. BBB has a similar function as in other vertebrates and it is composed of an endothelial cell layer, which promotes a tight junction with the presence of transport pumps. It is expected that bioactive compounds to be able to penetrate BBB and to exert central activity. The BBB plays a crucial role in protecting the central nervous system against xenobiotic [18,77,78]. Studies using compounds dissolved in water present some reason to believe the substance with CNS activity in fish might also penetrate BBB in mammals. However, there are several peculiarities that might limit the research because there is a substance that does not dissolve completely into water and thus a small amount of solvent must be used. It is not possible to control the chemical dose absorbed since zebrafish embryos, due to nondevelopment inside a placenta or in early life stages, are surrounded by a protective membrane which might limit the diffusion of some chemical compounds. Furthermore, substances can be metabolized in a different manner when compared to mammals [14,79-81]. Likewise, bioactive substances could display as potentially toxic in screening for unwanted and unexpected cardiovascular side effects. Although observing data on zebrafish regarding changes in shape and size during development, behavior, and heart rates simultaneously in a high-throughput and automated fashion is simple, they are valuable indicators of biotoxicity [82]. Because researchers can apply diverse phenotyping trials, zebrafish presents a good sensitivity and supports assays as a reliable, relevant, and efficient screening tool to identify and prioritize, an optimal model for toxicology, as well as in drug discovery research [83].

4. Gold nanoparticles like therapeutic agents in CNS

The use of nanoparticles (which are small molecules) has been increasing to explore a variety of applications in human health: they might become nanodrugs or help in the pharmacokinetic proprieties of drugs. Nowadays, the information produced from nanoparticles studies can help lead some risk of nanomaterials and nanotechnology related products. It may also contribute to the production of effective guidelines on designing strategies, protective measures and quality control tools to improve nanomaterials and minimize their toxicity [84,85]. Research to understand how specific nanoparticles interact with cells and cell systems are critical to evaluate their safety regarding either clinical or environmental exposure. In biomedical applications, manufacturing and other areas, nanoparticles are required to be verified as non-toxic. Cell lines and simple organisms are used for cell-level toxicity and genotoxicity studies, but vertebrate animals are necessary to analyze the complex physiological interactions [86,87].

Amongst various nanoparticles, gold nanoparticles (AuNPs) have shown important properties which are of full interest for pharmaceutical area such as drug delivery, cellular imaging, diagnostics, and therapeutic agents. The shape, size, and surface chemistry of AuNPs are the most common applications in a variety of biomedical ones [25,88,89]. The AuNPs are able to penetrate and move between biological compartments, hence it become potentially to use in human health. There have been concerns about potential hazards for human health from AuNPs considering their ability to penetrate and translocate in biological compartments [90,91].

Studies with AuNPs *in vitro* showed cellular toxicity, inflammation, and DNA damage, however, there is few *in vivo* toxicological studies concerned with AuNPs, and *in vivo* the evaluation of these models will be more predictive of potential hazard for humans [89,92]. Zebrafish model is employed as a model to check the toxicity of nanoparticles due to its low cost yet sophisticate, in addition to be very attractive to study *in vivo* nanotoxicity. Nanoparticles might be administered in a variety of different routes such as injection into eggs or specific sites on juveniles and adults; also, in water or as a sediment on food [3,10,11,72,88].

Pre-clinical trials could show the benefits of gold nanoparticles used in murine Parkinson model: the nanodrug enhanced symptoms such as motor coordination, and improved neuroinflammation *in vitro* [76]. Studies of antibacterial drugs show that the activation of gold nanoparticles with either nonantibiotic or antibiotic molecules might have a bactericide effect, assisting in fighting against bacterial resistance, besides suggesting some potential directions in the process of developing antibacterial drugs [93,94].

The use of gold nanoparticles to human health has been evaluated. Some studies used gold nanoparticles coupled with casein and were capable to penetrate the blood brain barrier of zebrafish larvae and eliminate the toxic amyloid protein and recovered the mobility and cognitive function of adult zebrafish exposed to amyloid beta protein. It is suggested that nanomedicine is safe and easy to use to eradicate toxic amyloid proteins implicated in a range of different human diseases [95]. Gold nanoparticles might interact with blood cells when are used as drug delivery carries. The nanodrugs delivery system was accumulated in leukocyte and mainly in platelet, which should be carefully evaluated [96].

5. Perspectives and conclusion

Nowadays, the information produced from nanoparticle studies can help to lead research about the risks of nanomaterials and nanotechnology-related products. It may also assist the development of effective guidelines on designing strategies, protective measures, and quality control tools to improve nanomaterials and minimize their toxicity. Zebrafish has a great potential for a positive impact on human health and medicine. Zebrafish have been used regularly in pharmacology and toxicology studies of drugs in early screening assay, due to the ease of raising them in a laboratory environment. Some studies using the zebrafish model for *in vivo* toxicity testing offered the efficiency to identify toxicity mechanisms. Zebrafish may be better used as a hazard identification tool and the model has been utilized for reproductive toxicology screening. The findings have proven useful to address issues related to CNS, cardiovascular, or gastrointestinal toxicity for several projects. The utility of outcomes shows a robust model with rapid throughput, low compound requirement, and low cost. Finally, it is noteworthy that because of the simplicity and speed of generating genetically engineered zebrafish models, this approach could be quite useful to interrogate via gene knockdown or knockout in an *in vivo*-like manner the safety

liability of targets of interest. Likewise, models that incorporate fluorescent labeling of specific cell types (e.g., vasculature, heart, pancreatic cells) could be quite valuable for pharmacological as well as toxicological assessment of compounds. In addition, it is growing the consensus that the use of zebrafish model might reduce the reliance on rodent testing for financial, ethical, and biological reasons [97]. Zebrafish show a great potential for pre-clinical trials, representing an ideal model to support the further development of nanoparticles as a medicinal agent. In general, zebrafish is a useful tool based on the evidence for translatable toxicology from zebrafish to mammals, and the animal model represents an optimum potential for toxicity and safety testing, besides being accepted by the Federal Drugs Administration for new drug approvals [18,98]. Gold represents an effective therapeutic agent for the treatment of some inflammatory disorders; however, it might become limited due to be associated to high incidence of side effects. Nanotechnology was able to coat gold with drugs thus improving the beneficial actions of it and reducing the toxic properties of these agents. Lower toxicity was reported with AuNP treatment, however, despite this therapeutic potential, safety of AuNP remains to be determined, since the balance between therapeutic properties and development of adverse effects is not well established. Zebrafish has already been exploited for gold nanoparticle toxicity studies, yet there is still an enormous potential for using in preclinical assays since prospected and future needs for research in this area are provided. These assays with AuNP in zebrafish could help create data that prioritize safer gold compounds for mammalian, disclose mechanisms of toxicity, and identify therapies that may mitigate the toxicity of promising therapeutics. The using of zebrafish for drug discovery is becoming one of the main organisms in translational neuroscience and psychiatry research, successfully complementing both rodent and clinical models of almost every major central nervous system disorder, and the assays might quickly and easily provide translatable data on a spectrum of tissues, organs, and systems.

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Conceptualization: Guilherme Carneiro Montes, Methodology: Guilherme Carneiro Montes, Software: Guilherme Carneiro Montes, Validation: Guilherme Carneiro Montes, Formal Analysis: Guilherme Carneiro Montes, Investigation: Guilherme Carneiro Montes, Resources: Guilherme Carneiro Montes, Data Curation: Guilherme Carneiro Montes, Writing - Original Draft: Guilherme Carneiro Montes; Writing - Review and Editing: Guilherme Carneiro Montes, João Eduardo de Moura, Visualization: Guilherme Carneiro Montes, Funding acquisition: Guilherme Carneiro Montes, Supervision: Guilherme Carneiro Montes, Project Administration: Guilherme Carneiro Montes.

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