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Integrating Zebrafish Research into Pharmaceutical Studies

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ABSTRACT:

Zebrafish are the source of several medicinal therapies that have lately made their way into clinical trials or the clinic. Because of their highly conserved molecular pathways, clinically relevant etiology, and disease features, zebrafish have become a potent preclinical model for human illness. They are also widely known for their contributions to developmental biology. When combined with cell-specific or tissue-specific reporters and gene editing technologies, zebrafish respond to small molecules and drug treatments at physiologically relevant dose ranges. This allows for the study of drug activity at single-cell resolution within the complex body of an animal, across tissues, and over an extended period. These characteristics allow for the discovery of new drug classes as well as the repurposing of existing medications for individualized and compassionate usage. They also enable high-throughput and high-content phenotypic drug screening. Drugs and drug leads that are investigated in zebrafish frequently have an inter-organ mechanism of action and would not be found by methods of focused screening. Here, we go over the significance of zebrafish as a model for drug discovery, the methods by which these findings are made, and upcoming changes to fully utilize zebrafish's potential for medical research.

Introduction

Zebrafish are the source of several medicinal therapies that have lately made their way into clinical trials or the clinic. Because of their highly conserved molecular pathways, clinically relevant etiology, and disease features, zebrafish have become a potent preclinical model for human illness. They are also widely known for their contributions to developmental biology. Zebrafish react to medications and small compounds at physiologically relevant dosage ranges.

In this Review, we go over recent developments in zebrafish disease modeling that have demonstrated the organism's potential for drug discovery and in vivo drug mechanism exploration, as well as its usefulness as a whole-animal model for preclinical drug repurposing and personalized medicine. Zebrafish have been a popular model organism for whole-animal chemical genomics and drug development because of their capacity for in vivo screening and the phenotypic' applicability to human disorders [1].

Aim and Objective

The aim is to Review zebrafish as a preclinical model In the sector of pharmacy for studying drug screening and also the effect of diseases and drugs. The objective to carry out the combined form of review of all the research done on zebrafish are:

1. To get aware of the use of drugs in human as well as fisheries medicinal science.
2. To establish the idea of using fish as a preclinical model for drug screening as compared to the other rodent animals used.
3. To familiarize with the established and broad importance of the pharmaceutical field in veterinary and fisheries science.
4. To study the health of fish by use of drugs in uncontrolled and test concentrations.
5. To know the interrelation between fish health and human health.
6. To get in touch with aquatic biodiversity and conservation of its ecosystem in medical or pharmaceutical ways.

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7. To draw the importance of industrial pharmaceutical vision in veterinary science by optimizing quality control status in animal husbandry science too.

Zebrafish

Known for their stripes, zebrafish (Danio rerio) are little tropical fish (2-4 cm) that inhabit rivers in southern Asia. Zebrafish were first used as model organisms in the laboratory to study developmental biology more than 40 years ago. Zebrafish are perfect for this since they can produce hundreds of embryos from a breeding couple, can be fertilized ex vivo, and are transparent, which makes it possible to track the development of a vertebrate from a single cell through organogenesis to a swimming zebrafish. Humans and zebrafish share 70% of the genome, and almost 80% of disease-causing proteins are conserved.[2]
Complex gene editing coupled with live high-resolution imaging has meant that development — as well as diseases — can be modeled and captured in unprecedented molecular detail and resolution[3]

Zebrafish illnesses are frequently accurate mimics of human diseases, capturing the genesis, course, and resolution of the disease[3, 4]. Zebrafish models of human disease alleles may be created by precision gene editing, and these models mimic aspects of the patient's etiology[5, 6]. Furthermore, it is frequently the case that genetic and transgenic models exhibit the same modifications in conserved underlying molecular pathways as those that underlie human disease[3,4]; transcriptional signatures and cancer cell states, for instance, are frequently shared between zebrafish models and human patients[7,8]. Zebrafish models have been created for a variety of diseases, including cancer[9], liver disease[10], blood disorders[11], heart disease[12], and behavioral disorders[13, 14]. Since zebrafish are vertebrates, their tissues and developmental biology are comparable to those of humans.
Since zebrafish and humans share many disease proteins and processes, medications that are effective in humans frequently have the same target in zebrafish, particularly those that interact with the active area of the target protein. This is particularly noticeable in medications intended to block cancer pathways, such as serotonin (5-HT) modulators[17], acetaminophen[16], and targeted therapies like MEK inhibitors [15, 16]. Generally speaking, compounds that are active in zebrafish are also active in mouse and human systems with similar pharmacokinetic properties[18]. However, there are some examples of drugs that work in humans but not in zebrafish, and vice versa. This information comes from more than 20 years of drug screening in zebrafish
Zebrafish are generally in use for preclinical studies as zebrafish show 80% physiological similarity towards human physiology as compared to other animals used for the same, this has been proved in many studies;

A. Zebrafish Gut:

There are striking similarities between the genes linked to human disorders and the genetic makeup of zebrafish. (19) The schematic for the same is shown under Fig. (i) in the section titled "List of Tables and Figures." There are genetic similarities between zebrafish and humans. Because of this, it would be simple to evaluate all of the data by the methodology used for the research on the zebrafish gut.
Complex behaviors exhibited by zebrafish are comparable to certain facets of human behavior, including social interaction, learning, and reaction to environmental cues.[25]Drug impacts on behavior and neurological function may be investigated using behavioral tests in zebrafish models, which can provide details about the underlying neural systems.[25] Overall, zebrafish are useful models for researching human biology, disease processes, and treatment responses because of their genetic and physiological similarities, even if they vary from humans in other aspects, especially in terms of morphology, physiology, and complexity. Their fast rate of reproduction, optical transparency throughout embryonic development, and genetic modification susceptibility further augment their usefulness in biological research.[23, 25]

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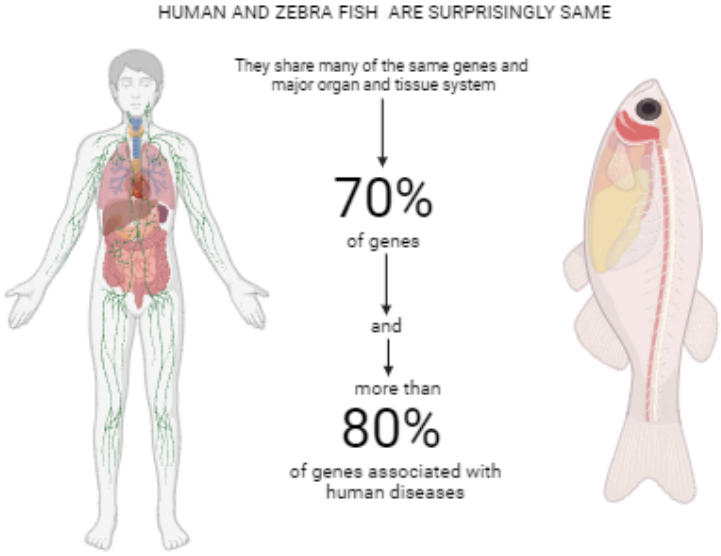

Fig (i): Genetic similarities between human and zebrafish

B. **Model of Zebrafish for Research**:

Clinical science has benefited greatly from the pathological, genetic, physiological, and biological discoveries made in zebrafish models for the study of concurrent metabolism and metabolic diseases in advanced eukaryotes. (20) This is a result of the zebrafish's gut's physical similarities to human anatomy. (20)
The general characteristics of zebrafish in clinical research sciences are as follows; (20)

- Rapid external development
- Optical transparency of the fertilized embryo
- Tractable genetics
- Specific gut microbiota
- Ease of maintenance

C. **Zebrafish Gut Anatomy**:

To comprehend the zebrafish's gut's precise structure, Fig. (ii): The illustration of zebrafish (larval) gut anatomy can be found in the "List of Tables and Figures" section. (20) During their larval stage of development, zebrafish adopt the body shape of a typical higher vertebrate. (20)

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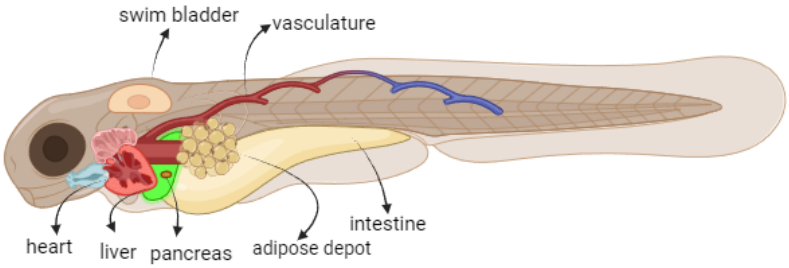

Fig. (ii): The illustration of zebrafish (larval) gut anatomy

D. Correlation Between Effect of Drugs on Zebrafish Gut and Human Beings:

As previously indicated in point (B), humans and zebrafish share a strikingly similar genetic makeup. (19) This presents a compelling case for connecting the findings and conclusions of human health research. There are two methods to estimate this interlinking:

1. The effects of drug pollutants present in the aquatic environment on the gut of zebrafish and the effect of the same on the gut of humans.

2. A specific effect on the gut health of zebrafish can be assumed consecutively with the same effect on the health of the gut of other fishes too, which may give alarming results for human health, especially in a population that feeds on fish in their dietBecause of their many genetic and physiological parallels to humans, zebrafish (Danio rerio) are useful models in the study of human biology and disease. The following are some significant similarities between humans and zebrafish:

Genetic Homology:
Humans and zebrafish share a large amount of genetic material. Studies have revealed that zebrafish orthologs for over 70% of human genes that code for proteins exist. Zebrafish and humans share several essential signaling pathways and biological processes, including those related to development, immunity, and metabolism. With the complete sequencing of the zebrafish genome, comparative genomics research can now be used to determine which genes and regulatory elements are evolutionarily conserved in both zebrafish and humans.[21]

Organ Structure and Function:
Human organs and tissues, such as the heart, brain, liver, pancreas, and kidneys, are anatomically and functionally comparable to those found in zebrafish.[21]The study of organ development, function, and disease is made easier by the striking parallels between the cellular architecture and physiological processes of these organs in zebrafish and humans.[21]

Embryonic Development:
Zebrafish embryos develop quickly, and they are similar to human embryos in many developmental stages and molecular pathways. Because of their transparency, zebrafish embryos are especially helpful for researching embryogenesis and organogenesis because they provide real-time viewing of developmental processes.[22]

Disease Modeling:
Through genetic manipulation, illness models of several human ailments, such as cancer, cardiovascular disease, neurological disorders, and metabolic syndromes, may be created in zebrafish.[23]It has been shown that many illness-associated genes and pathways uncovered in zebrafish models are retained in humans, offering important new information about the processes underlying disease and possible targets for treatment.[23]

Drug Responses
Zebrafish are valuable models for pharmacological research, toxicity testing, and drug development because they react to pharmaceuticals similarly way humans. In high-throughput drug screening tests, zebrafish models may be used to find possible medicinal compounds and assess their safety and effectiveness characteristics.[24]

Journal of Chemical Health Risks

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JCHR (2024) 14(3), 1587-1598 | ISSN:2251-6727

Behavioral Responses:

Complex behaviors exhibited by zebrafish are comparable to certain facets of human behavior, including social interaction, learning, and reaction to environmental cues.[25]Drug impacts on behavior and neurological function may be investigated using behavioral tests in zebrafish models, which can provide details about the underlying neural systems.[25] Overall, zebrafish are useful models for researching human biology, disease processes, and treatment responses because of their genetic and physiological similarities, even if they vary from humans in other aspects, especially in terms of morphology, physiology, and complexity. Their fast rate of reproduction, optical transparency throughout embryonic development, and genetic modification susceptibility further augment their usefulness in biological research.[23, 25]

There are many experimental studies carried out by using zebrafish as a preclinical model for analyzing the action of drugs for particular diseases; they are

(A) Zebrafish as the model used for inflammation study

The study injected carrageenan into the coelomic cavity, observing a significant increase in total leukocytes at concentrations of 3.5% and 1.75% compared to the control group. The concentration of 3.5% showed a significant increase at all times compared to the control group. The NAG enzyme activity was also evaluated, showing that the concentration of 3.5% Cg significantly increased NAG activity at 1, 2, 4, 5, and 6 hours after stimulation compared to the group receiving the vehicle. The liver, intestine, and mesenteric were analyzed for microleaks. The livers of animals injected with the vehicle alone had hepatocytes with a polygonal shape and minimal leukocyte infiltrates. In zebrafish, carrageenan increased the frequency of hepatocytes with cytoplasmic vacuolation and numbness, decreased eosinophilia, and led to an infiltration of mononuclear cells near the vascular endothelium. The mesentery in the coelomic cavity showed connective tissue rich in adipose cells and dispersed pancreatic acini clusters. The study also investigated the expression of inflammatory genes induced by carrageenan in a zebrafish model.

(B) Zebrafish as the model used for antimicrobial activity

The study analyzed Rajath Bhasma, a plant used in experiments, using techniques such as FTIR, SEM, EDX, DLS, and XRD to determine surface functional groups, particle size, shape, and crystalline phases. The study assessed embryonic toxicity using zebrafish embryos treated with Rajath Bhasma at different concentrations for 48 hours. The antimicrobial activity of Rajath Bhasma against gram-positive and gram-negative bacteria was determined using the well-diffusion method. Rajath Bhasma, a silver nanoparticle, was characterized using these techniques. The broad peak at 3,433 cm−1 corresponds to an H-bonded hydroxyl group, attributed to the preparation method using plant compounds. The average crystallite size of Rajath Bhasma is approximately 55 nm. The toxic effects of Rajath Bhasma on zebrafish embryos were determined, showing that 5 µg/ml did not substantially inhibit zebrafish embryo growth. The study confirmed that silver is the major component of Rajath Bhasma, and XRD analysis identified crystalline phases. Rajath Bhasma was found to be an effective antimicrobial agent without toxicity when used at low concentrations.

(C) Zebrafish as the model used for antioxidant activities

The study investigated the antibacterial and antioxidant properties of copper sulfide nanoparticles. The nanoparticles were tested against various pathogenic bacteria and their antioxidant activity was assessed by assaying the free radical scavenging effect on DPPH. The study also evaluated the anti-inflammatory effects by measuring the percentage of inhibition in human red blood cells.

The toxicology of synthesized copper nanoparticles was analyzed using zebrafish embryos. Healthy embryos were placed in 96-well culture plates with different concentrations of CuS nanoparticles (0 to 150 µg/mL). The developmental status of the embryos and larvae was observed at different fertilizing periods. Hatching and mortality rates were calculated every 12 hours. The UV-vis absorption spectrum of copper sulfide nanoparticles was used to study their optical properties and bioreduction. The EDX graph showed the elemental composition of the nanoparticles, with a strong signal at

Journal of Chemical Health Risks
www.jchr.org
JCHR (2024) 14(3), 1587-1598 | ISSN:2251-6727

1 keV assigned to elemental copper and a weak signal at 2.3 keV displaying the sulfur component. CuS nanoparticles showed antibacterial activity against gram-positive and gram-negative bacteria, including E. coli, Vibrio harveyi, Vibrio parahaemolyticus, Bacillus sp., and Proteus sp. The highest zone of inhibition was observed in E. coli at the highest concentration, while the highest antioxidant activity was observed at the maximum concentrations.
Embryos were treated with different concentrations of CuS nanoparticles, and developmental abnormalities were observed. The higher concentration of CuS nanoparticles caused developmental toxicity and growth retardation in zebrafish.

(D) Zebrafish as the model used for antimicrobial activity

The study involved double Tg(mpeg1:mCherry/mpx:eGFPi114) zebrafish larvae exposed to different concentrations of saponin. The larvae were anesthetized and imaged using fluorescent microscopy at 24 and 72 hours after immersion. The study also assessed the effect of antibiotics on the larvae, with four pools of 5 larvae sampled at 6 days post-infection. DNA was isolated from three pools of 5 larvae to investigate microbiome composition at baseline The study aimed to assess changes in gene expression in larvae using total RNA isolated from pools of larvae. The results showed that saponin exposure decreased survival in a dose-dependent manner, increased neutrophil recruitment to the intestinal area, and increased pro-inflammatory and anti-inflammatory cytokine expression.
Zebrafish larvae exposed to either ciprofloxacin or oxytetracycline showed slightly lower il1b expression but no alteration in neutrophil and macrophage recruitment to the intestinal area. However, oxytetracycline did not reduce saponin-induced neutrophil recruitment to the intestinal area upon co-treatment. Antibiotic treatment alone or in combination with saponin did not show significant changes in gene expression.
The study used Redundancy Analysis (RDA) to understand the microbiome shift in zebrafish larvae exposed to saponin and oxytetracycline. The top 25 most discriminating OTUs were correlated with the treatment groups, indicating that the microbiome shift was saponin but also antibiotic treatment dependent.

(E) Zebrafish as model used for antiviral activity

Male adult zebrafish were divided into two tanks, one with antibiotic treatment (SMX and CLA) and the other with DMSO. After two weeks, half of the fish were anesthetized and intraperitoneally infected with SVCV sublethal dose. Four fish from each condition were sacrificed, and the intestine and kidney samples were stored at -80°C. The remaining fish were exposed to DMSO or antibiotics for mortality monitoring The study analyzed the bacterial and fungal profiles of fish exposed to antibiotics in their intestine and kidney. The majority of bacteria were found in the intestine, while the most abundant fungi were found in the kidney. The most abundant bacterial classes were found in the intestine of DMSO-treated zebrafish, with the most abundant class being Alphaproteobacteria. No significant differences were observed in the relative abundance of bacterial and fungal classes in kidney samples from DMSO- and antibiotic-treated fish. A study involving adult zebrafish exposed to 0.01 mg/L SMX and CLA and infected with SVCV showed a 100% survival rate for the DMSO-SVCV group, while a significantly lower survival rate was observed for the combination of both antibiotics. High-throughput transcriptome sequencing and RNA-Seq analyses showed a clear differentiated distribution between DMSO- and antibiotic-treated fish, while kidney samples were more randomly distributed. The study suggests that antibiotic treatment may have a stronger effect on the intestine than on the kidney

(F) Zebrafish as the model used for probiotic effects

The study aimed to measure the height and weight of Zebrafish and record their mortality rate. Tree fish was collected from each treated group and dissected under a biological hood and aseptic condition. Samples were dehydrated, infiltrated, and sectioned by microtome and sectioned by microtome. Lams were prepared for investigation by fluorescent microscopes. Three fish were collected from each treated group and examined under fluorescent dying for the detection of probiotics in intestinal tissues and spatial distribution.
The study quantitatively assessed the expression

Journal of Chemical Health Risks
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JCHR (2024) 14(3), 1587-1598 | ISSN:2251-6727

levels of probiotic bacteria in intestinal tissues using PCR assays. Three Zebrafish were collected from each treated group on the 28th, 56th, and 60th day of the assay. The results showed no significant differences in the normal group compared to its initial form. However, the treated groups showed longer villus compared to the control groups. The number of goblet cells increased in all parts of the intestine compared to the control group. After 56 days, there was an increased rate of goblet cells in proximal, middle, and distal sections in intestinal tissues of Zebrafish models with a significant difference among G3 & G4 and control groups (G1). The most abundant existence of probiotics was found in intestinal tissues of Zebrafish models infected by Aeromonas hydrophila receiving both prebiotic bacteria (G4) compared to control and zero groups.

(G) Zebrafish as model used for multi-drug resistance

Zebrafish larvae were mechanically dechorionized, anesthetized, and injected with Salmonella strains into Cuvier's duct. Antibiotics were injected at specific time points, and the results were analyzed. Using confocal-laser scanning microscopes, the study determined the appropriate number of colony-forming units (CFU) for drug screening in a ZFL. Survival was dependent on the dose, with doses higher than 5000 CFU leading to rapid death. The ZFL Blood-Infection Model was validated by examining the impact of ceftriaxone and tobramycin treatment on ZFL infected with Salmonella. Ceftriaxone showed high survival rates and minimal bacteria in circulation and macrophages, while tobramycin showed poor cell penetration. The study validated ZFL as a model for determining extra- and intracellular antimicrobial activity in live animals. A model was tested using ZFE embryos infected with DNA-damage reporter Salmonella, showing detectable mCherry but no GFP signals. Three fluoroquinolones were tested against Salmonella, and the results showed excellent cell penetration, making them suitable for treating systemic Salmonella infections in humans.

(H) Zebrafish as the model used for genotoxic impact

The study investigates the toxicity of amoxicillin residue (AMX) in zebrafish embryos. The maximum residue concentration was determined using HPLC and livability under 1xMRL inoculums for 24 hours. The concentration of AMXR was found to be 1066 X 1 MRL in acetonitrile in a pasteurized milk sample, with a tolerance of up to 2 X MRL. The study administered treatments to 48-hour-old zebra fish embryos, monitoring their development using the Comet Assay. The results showed that AMXR was present in raw and pasteurized milk, with a higher quantity in raw and pasteurized milk. The study found that AMXR had adverse effects on zebrafish growth and development, with the AMXR-treated group showing reduced body length compared to the untreated group. The study also found a substantial increase in the tail moment of treatment with AMXR compared to the untreated classes, ACTN (0.23) or AMX (0.35). These findings highlight the potential dangers of using amoxicillin in raw and pasteurized milk and the potential for further contamination.

(I) Zebrafish as the model used for microbiota

The study analyzed zebrafish intestine samples using PCR and clone libraries. The samples were euthanized, dissected, and combined with Zirconia/silica beads, Na-phosphate buffer, and lysis solution. The resulting DNA was analyzed using PCR and the CEQ8000 Genetic Analysis System software. The mapping file and operational taxonomy unit (OTU) table were used to generate non-phylogenetic diversity metrics. The clone library sequences were analyzed using the SINA aligner manually evaluated in MacClade 4.06, and imported into the ARB database dendrogram. The study also analyzed three pooled intestinal samples using massively parallel barcoded pyrosequencing. The resulting clone library sequences were taxonomically classified using the RDP-II Naïve Bayesian Classifier version 2.2. The researchers found that gut bacterial communities in both domesticated and recently caught zebrafish have similar structures. The taxonomy of intestinal bacterial communities in zebrafish and other fish species was compared, with two bacterial classes, g-Proteobacteria and Fusobacteria, consistently appearing in the gut microbiotas of zebrafish and other fish species

Journal of Chemical Health Risks

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JCHR (2024) 14(3), 1587-1598 | ISSN:2251-6727

(J) Zebrafish as the model used for antibiotic-associated diarrhea

The study investigated the toxicity of crude protein extract on adult zebrafish using a 96-hour test time. The animals were divided into three groups: control, Amoxicillin diet, and Amoxicillin treated with different concentrations of crude protein extract. Mortality was monitored continuously, and the fish were considered dead when operculum movement and response to mechanical stimulation could no longer be detected. Histopathological examination of the intestinal segments of the sacrificed fish was also conducted. The study identified bacterial isolates with a 99.87% similarity to Aneurinibacillus migulanus BJ-44 and Aneurinibacillus sp. YR247. In vitro, the evaluation of probiotic characteristics showed a survival rate of visible growth. Bacillus sp showed higher enzymatic activity on casein-induced nutrient agar medium. The study also examined the acute toxicity of crude protease extract on antibiotic-induced zebrafish. The crude protein extract showed higher weight gain in antibiotic-treated groups compared to the control group, indicating its therapeutic significance. Histopathology studies showed a high degree of restoration, with villi and goblet cells prominent. Molecular docking studies showed that the crude protein extract inhibited Clostridium difficile Toxin A, a pathogenic toxin. The study highlights the therapeutic significance of the intracellular protease extract in treating antibiotic-induced zebrafish..

(K) Zebrafish as the model used for behavioral change by drugs

The study involved 6-9 months old adult wild-type zebrafish from a breeding colony, treated with antibiotic drugs based on their maximum concentrations in water bodies, toxicity, and behavioral studies. The animals were exposed to three concentrations of Chlortetracycline, Ciprofloxacin, or Ceftazidime in tank water for 96 hours, while the control group remained unaffected. Behavioral experiments were conducted in water tanks with adjusted water conditions, and the recordings were analyzed using Any-Maze Software for behavioral parameters. The animals were placed in a Y-maze glass aquarium with three arms, and their social behavior and innate preference for conspecifics were analyzed. All antibiotic drugs influenced locomotor behavior, with animals treated with Ciprofloxacin, Ceftazidime, or Chlortetracycline showing increased distance traveled compared to the control group. Memory impairment was observed in the Y-maze test, but only 6.25 mg/L for each antibiotic was evaluated for aggression and social interaction. Antibiotic exposure altered aggressive behavior, with Ciprofloxacin-treated animals spending more time in the segment nearest to the mirror image.

(L) Zebrafish as the model used for studying pathogen susceptibility

The study examined the impact of low-concentration antibiotic chronic exposure on the gut microbiome of zebrafish. The experimental model was cultured under laboratory conditions and exposed to various antibiotics for 30 days. The gut microbiome composition was confirmed at the phylum level, with Fusobacteria being the major phylum. However, alteration of the gut microbiome composition at the phylum level was confirmed in zebrafish chronically exposed to antibiotics. The relative abundance of Gram-positive bacteria at the genus level rapidly decreased in the erythromycin-treated zebrafish gut microbiome. The relative abundance of Cetobacterium somerae was significantly decreased in the Smx/Tmp and Ery groups, while Aeromonas veronii, an opportunistic infection bacteria, was significantly increased in the Ery group. In the beta diversity analysis, dysbiosis of the gut microbiome occurred in all zebrafish treated with antibiotics, with the erythromycin-treated zebrafish exhibiting the greatest degree of dysbiosis. The study also investigated the regulation of intestinal immune and stress-related gene expression in fish using qRT-PCR.

(M) Zebrafish as the model used for bacterial resistance after antibiotic exposure

The study aimed to investigate the effects of antibiotic use on the abundance of tetracycline-resistant bacteria in zebrafish skin and water. The researchers selected 60 antibiotic-resistant isolates from samples exposed to the highest concentration of oxalic acid (10 µg/mL). The study used PCR to inspect the occurrence of tetracycline resistance genes and analyzed their antibiotic susceptibility profile using the disk diffusion method on

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Mueller-Hinton Agar.
The results showed that the presence of tetracycline-resistant bacteria was higher in water and fish skin exposed to oxalic acid compared to control samples. After exposure ceased, the total counts of resistant bacteria increased, indicating the selection of ARB due to antibiotic use. The study identified 60 isolates from each culture medium, with 37% identified as Stenotrophomonas and 63% as Pseudomonas. Stenotrophomonas and Pseudomonas are pathogenic to fish and humans due to their low membrane permeability, efflux pumps, and ability to biodegrade tetracyclines.
Zebrafish embryos were used to study the pathogenicity of seven multidrug-resistant Stenotrophomonas strains. All strains induced mortality in zebrafish embryos, but differences were not statistically significant for five strains. Only two isolates showed significant results, one from water and one from fish skin. The infection via immersion tests the capacity of a pathogen to cross the fish's natural barrier, simulating the natural exposure pathway. However, it is impossible to determine the exact number of bacteria that can invade the organism. Future studies using other endpoints like fish immunological response or strains' virulence factors may provide a deeper understanding of the pathogenic potential of these isolates. The tetA gene, a frequently detected tetracycline resistance gene, was detected in both zebrafish gut and water samples, irrespective of antibiotic exposure. The results indicate that OTC may select ARB and promote the increase of ARG abundance, namely tetA, although this increase is exposure time-dependent. The selection of tet resistance genes may also allow the co-selection of ARGs to other antibiotics.

(N) Zebrafish as the model used for discovering novel bioactivities

The study involved zebrafish wildtype strain AB, maintained in a Techniplast rearing system under a 14:10-hour light/dark photocycle. After breeding, eggs were collected and fertilized embryos were distributed into 6-well plates containing 5 mL of E3 medium supplemented with appropriate concentrations of test compounds. Embryonic mortality and morphology rates were monitored, and dead embryos were removed. The experiments tested six to nine concentrations chosen following a range-finding test. Statistical analysis was performed using mixed effects logistic regression, and no observed adverse effect concentrations (NOAECs) were determined from the tested concentrations.
In silico analysis using online tools was performed to obtain a first hint concerning possible biological processes involved. Two FAs induced substance-specific phenotypes: Quinoline Yellow (QY) caused swollen yolk, and Brilliant Blue (BB) induced. Sodium Benzoate (SB) was found to be a potential teratogen with a "Teratogenic Index" (LC50/EC50) TI >1. Quinoline Yellow (QY) was found to be extremely teratogenic with a TI~79
A study found that benzoic acid (SB) is the most aquatoxic free amino acid (FA) with an LC50 of 26.9 mg/L. SB has been shown to induce a darkened yolk phenotype at concentrations as low as 5 mg/L, suggesting nephrotoxic activity. Consumption at 100-500 mg/kg bw/day has been shown to induce kidney damage in Wistar rats and mice. SB is extensively used in food and cosmetic products, with the European ADI for SB being 5 mg/kg bw. The study emphasizes the need to reconsider SB's safety levels in food and cosmetic products, especially during pregnancy and childhood.

(O) Zebrafish as model used for environment risk assessment of drugs

The study investigated the optimal concentrations and time points for embryonic development experiments using dexamethasone sodium phosphate (DEX) and Tocilizumab (TCZ) in zebrafish. The researchers used wild-type mature zebrafish, raised in Italy, and monitored the toxic effects of DEX and TCZ in combination. Mortality and developmental abnormalities of embryos and larvae were monitored at 24, 48, 72, and 96 hours post-fertilization.
The study also examined the viability and morphology of zebrafish embryos after preliminary exposure to DEX and TCZ. DEX concentrations of 0.1 and 1 µmol/L did not alter zebrafish morphology after 96 hours, while DEX concentrations of 5 µmol/L increased mortality. TCZ doses of 221.05 and 442.1 µmol/L did not affect zebrafish morphology after 96 hours.
The study found that co-exposure of DEX and TCZ increased SOD and CAT expression levels related to oxidative damage in larval zebrafish, and significantly increased MDA levels at 96 hours post-exposure. The protein levels of apoptosis-related genes (caspase-3 and bax) rose following DEX 1 µmol/L + TCZ 442.1 µmol/L

Journal of Chemical Health Risks

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JCHR (2024) 14(3), 1587-1598 | ISSN:2251-6727

treatment, while bcl-2 expression was downregulated. The results suggest that DEX + TCZ induces cell death in zebrafish embryos

Conclusion

In conclusion, the zebrafish is a highly helpful vertebrate animal model for drug development and scientific studies. Specifically, functional validation of candidates in zebrafish is significantly improving the capacity and accuracy of identifying causative genes and molecular mechanisms underlying the pathogenesis of human genetic diseases, thanks to the use of CRISPR-based knockout technology and big data from next-generation DNA sequencing. These initiatives are essential to building the foundation for precision medicine's future since they offer fresh molecular targets for treatment and diagnostic approaches, particularly those about uncommon illnesses.

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