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A BLESSING IN DISGUISE: FROM FISH VENOM TO NOVEL MEDICINES

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YOUNG REVIEWERS:

COLLEGE HILLS ELEMENTARY

AGES: 8–9

VEDANT

AGE: 8

Every day, scientists make discoveries that help improve human health and wellness. Many medicines are discovered in nature, like toxins from animals. We decided to study a fish called Copper Joe toadfish (*Thalassophryne nattereri*) and found that a small molecule in the fish's venom, called *Tn*P, can help damaged organs and tissues. However, before any molecule can be used in humans, it must be tested on laboratory animals. These tests are called pre-clinical trials and are important to prove that a treatment is safe for humans. Our study tested *Tn*P in zebrafish and found that it is safe and has no toxic effects on the developing fish. In the future, scientists can continue researching *Tn*P, and hopefully, someday, it will be developed into a medicine to help thousands of people around the world who are suffering from diseases that cause inflammation.

SOME FISH ARE POISONOUS

INFLAMMATION

A response of the body to harmful stimuli, diseases, or infections by bacteria or viruses. Redness, heat, swelling, and pain are common symptoms during inflammation.

Did you know that there are many kinds of poisonous fish? Would you believe that the venom of one of these fish contains a substance that can treat diseases that cause **inflammation**? It is true! The venom present in poisonous fish is a mixture of many molecules. The fish use these important molecules to defend themselves from their enemies (other animals that want to feed on them) and to protect the areas where they live, by scaring away invaders to defend their territory.

Thalassophryne nattereri is the scientific name of a poisonous fish in Brazil, which causes many incidents involving fishers and sunbathers. It is mainly found in the north and northeast regions of Brazil and is popularly known as niquim, or Copper-Joe toadfish. The niquim is a small fish, measuring about 15 cm in length; it likes to spend most of its time buried in the sand in brackish (slightly salty) water, where rivers meet the sea. This venomous fish has four spines, one on each side of its body, and two on the top. These spines are naturally hollow, like straws, and when the spines are pressed, poison from venom glands comes out of them (Figure 1). Incidents can happen when people are walking along the beach, swimming in the sea, or even when the fish get caught on fishing hooks or in nets. If a person steps on a niquim or accidently touches the spines, the poison comes out of the fish and goes into the human. These incidents cause extreme pain, and the region where the poison enters becomes swollen and red.

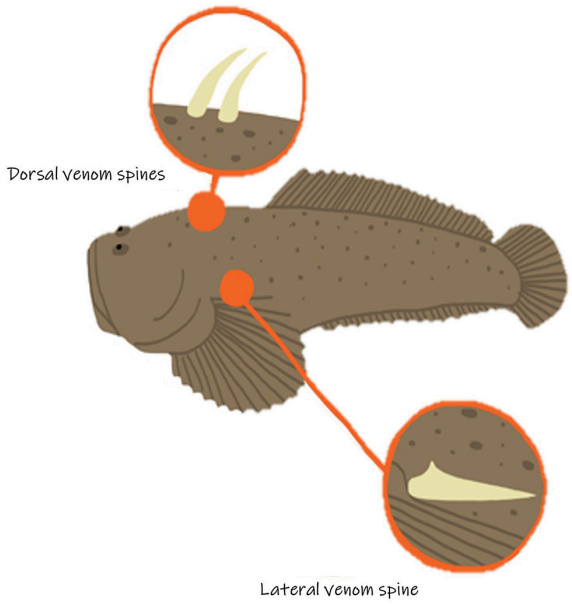

Figure 1

The fish *Thalassophryne nattereri*, commonly called the niquim in Brazil, has a venom system composed of four hollow spines (like needles): two on the top of the body and one on each side. The spines are connected to venom glands, which produce venom and are found at the base of the fins. When a person touches this fish with their hands or steps on it, the spines enter the skin. This presses on the venom glands, which then release the poison into the person's body.

Within a week the tissue around the wound starts to die, becoming **necrotic** [1].

Our group has been studying the niquim and its venom since 1996, and we have made many important discoveries. We were the first to identify the main molecules present in the venom. We named them **nattectin** and **natterins**. Natterins are a set of proteins (there are five of them), but the venom only has one nattectin protein. Together, these molecules are responsible for the pain, swelling, and necrotic tissue that develop in humans that have been in contact with the fish's venom. Interestingly, we recently discovered that natterins are present in many other organisms, and their function is to protect those organisms [2].

A GREAT DISCOVERY!

We discovered another important small molecule in the venom of the niquim—a **peptide** that we named **TnP**, for Thalassophryne *nattereri* peptide. Unlike natterins and nattectin, we found that *Tn*P was not involved in the symptoms observed in people that have niquim incidents. In fact, our studies showed that *Tn*P inhibited inflammation. Let us remember that inflammation is a response of the body to harmful stimuli, diseases, or infections by bacteria or viruses. The finding that *Tn*P inhibited inflammation was a very interesting discovery, so we did additional studies to find out more about *Tn*P.

First, we examined *Tn*P's effect in animals. We started with mice, and those experiments helped us discover that *Tn*P decreases inflammation caused by two critical diseases that many people worldwide suffer from: asthma and multiple sclerosis. Asthma is a disease of the respiratory system, and multiple sclerosis is a disease that affects the central nervous system. *Tn*P proved effective in protecting against the development of these diseases, relieving their symptoms, and did not cause side effects.

FROM THE LAB TO A MEDICINE

Our results increased our confidence that *Tn*P could be used as a medicine to treat inflammatory processes, but first it was necessary to investigate its safety—for a substance to become a medicine, it must be proven to be safe for humans. This safety testing, called a **pre-clinical study**, is also performed in animals. The pre-clinical study is an essential step in developing a new drug (Figure 2).

For our pre-clinical study, we used zebrafish, a little fish native to South Asia and that shares 70% of its genes with humans and is currently widely used as an experimental laboratory animal for drug

NECROTIC

When most or all of the cells in an organ or tissue die due to disease, injury, or failure of the blood supply.

NATTECTIN

A kind of lectin protein isolated in the niquim venom that binds to sugar molecules (carbohydrates) and is involved in inflammation.

NATTERIN

New proteins discovered in the venom of the fish *Thalassophryne nattereri*, which are the most abundant proteins in the venom and the main responsible for the envenomation symptoms and wounds in humans.

PEPTID

Short chains of amino acids, the same parts that together form the proteins that play critical roles in the biological functions of all kinds of organisms.

TNP

Thalassophryne nattereri peptide, a peptide discovered in the venom of the niquim that helps to treat inflammation.

PRE-CLINICAL STUDY

Research using laboratory animals to determine if a drug, procedure, or treatment is effective and safe before it is tested on human volunteers.

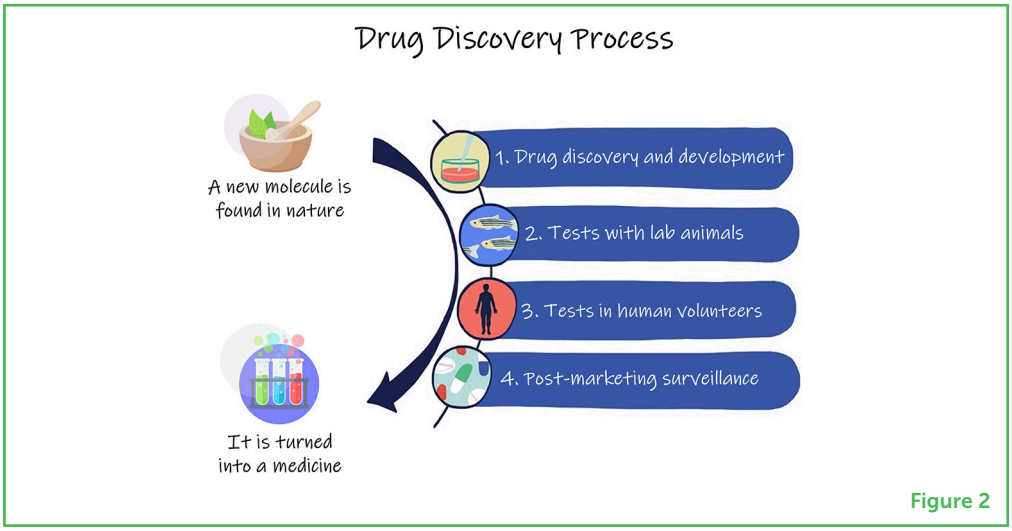

Figure 2

To develop a new drug, scientists often find and isolate interesting molecules from nature. These substances must be tested on experimental animals (like zebrafish or mice) to confirm that they are safe and effective for treating diseases—these tests are called pre-clinical studies. When enough is known about the substance, it can be tested on human volunteers. Once the medicine has passed testing and is available in pharmacies, there is continuous monitoring to check for any kind of problems in people using it.

development [3]. Zebrafish have numerous advantages, including rapid growth, a transparent **embryo-larval stage**, and a small size [4].

*Tn*P IS SAFE AND EFFECTIVE

To ensure the safety of *Tn*P, we first exposed groups of zebrafish embryos to various doses of *Tn*P. We monitored the embroys/larvae every day for 4 days to look for any defects in development. For comparison, we examined a separate group of fish embryos that were not exposed to *Tn*P (Figure 3). Then, we checked whether the hearts of the zebrafish larvae were beating correctly, whether the larvae were swimming normally, and whether the larvae' brains were well-developed [5]. All these tests confirmed that *Tn*P did not cause heart or brain damage in the embryos that were exposed to it.

EMBRYO-LARVAL STAGE

The earliest developmental stages of fish. The embryo stage includes development in the egg, while the larva stage occurs right after the fish leaves the egg and starts to swim.

CONCLUSION

Our studies allowed us to conclude that *Tn*P, a molecule from the venom of the fish niquim, is safe and effective and could be a promising drug for the treatment of human diseases that involve inflammation, such as asthma and multiple sclerosis. The next step is continuing testing *Tn*P to better understand how does it helps in the immune response, and clarify its mode of action. In the future, we will continue to work with *Tn*P, evaluating its ability to inhibit the inflammatory process of other important human diseases.

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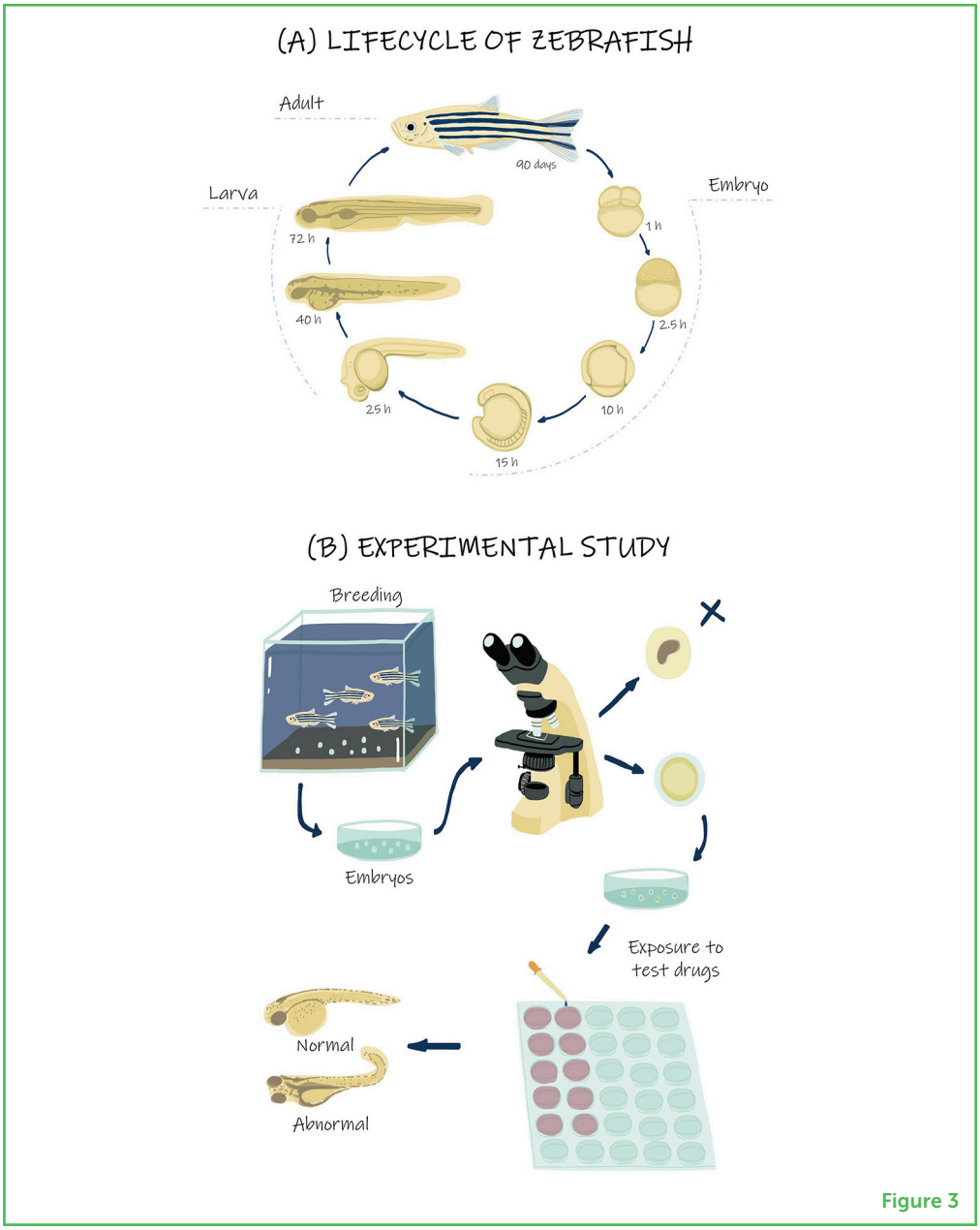

Figure 3

(A) Zebrafish development occurs very quickly. They grow as much in a day as a human baby grows in 1 month. Most organs are functional 72 h post-fertilization. **(B)** Since zebrafish embryos and larvae are nearly transparent, researchers can examine the development of their internal organs to look for toxic effects caused by exposure to test drugs. Zebrafish and humans are vertebrates—their muscles, blood, kidneys, and eyes share many features, which makes zebrafish a good model for studying some human diseases.

dissemination branch. Also, huge thanks to the Zebrafish Platform staff and collaborators. Special thanks to the young reviewers and mentors for the meaningful feedback.

ORIGINAL SOURCE ARTICLE

Batista-Filho, J., Falcão, M. A. P., Maleski, A. L. A., Soares, A. B. S., Balan-Lima, L., Disner, G. R., et al. 2021. Early preclinical screening using zebrafish (*Danio rerio*) reveals the safety of the candidate anti-inflammatory therapeutic agent *Tn*P. *Toxicol. Rep*. 8:13–22. doi: 10.1016/j.toxrep.2020.12.004

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CONFLICT OF INTEREST: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

YOUNG REVIEWERS

COLLEGE HILLS ELEMENTARY, AGES: 8–9

We are a group of 3rd Grade "Gifted and Talented" students at College Hills Elementary School in College Station, Texas. It is a Dual Language, Title I campus with a long history. We are a diverse group of friends who love to learn, tackle

hard challenges, and have fun. We enjoy STEAM projects with LEGO and really like chocolate! We learned about many scientific topics while reviewing this article and had fun sharing our thoughts. We did like to do it again!

VEDANT, AGE: 8

I like playing with legos and minecraft. I like asking questions and exploring things around me. I am always curious.

AUTHORS

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Mônica is a biologist with a Ph.D. in immunology from the University of São Paulo. She did postdoctoral studies in biochemistry and pharmacology at the Butantan Institute. In 2004, she joined the Butantan Institute as a scientific researcher and began studying venomous fish. Since 2010, she has been a principal investigator in the CEPID/FAPESP project, coordinating Plataforma Zebrafish™. Mônica studies the toxins of venomous fish, the immune response, and also use zebrafish as a model to study the risk of environmental contamination. She shares scientific knowledge with society through various projects, many of which are carried out in public and private schools. *monica.lopesferreira@butantan.gov.br

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Rodrigo is a Brazilian biologist with a Ph.D. in genetics whose research has focused on studying how natural substances or chemical contaminants affect the health of organisms, especially fish. Recently, he has been investigating animal toxins and their roles as novel medicines, mainly using zebrafish as an experimental model. Rodrigo is very passionate about nature and wildlife and enjoys spending time outdoors.

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Maria is a pharmacist who graduated from the Federal University of Alagoas, with a master's in health sciences specializing in clinical pharmacology, clinical and hospital pharmacy. She has a Ph.D. in pharmaceutical sciences from the Federal University of Sergipe and Butantan Institute. She has performed pre-clinical testing involving mice and zebrafish to study pain, inflammation, gene editing, and toxicity. Maria is currently a pharmacist at the Drug Information Center at Clinical Hospital of the University of São Paulo Medical School, working in evidence-based health, clinical research, and mentoring.

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Carla is a pharmacist with a Ph.D. in immunology and postdoctoral work in pharmacology from the University of São Paulo. She has been a scientific researcher at the Laboratory of Applied Toxinology since 2004, and she has been co-founder and deputy coordinator of the Plataforma Zebrafish™ since 2015. She does drug-development research in association with Brazilian pharmaceutical industries, including pre-clinical studies using mice and zebrafish. Her research mainly focuses

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[†]These authors have contributed equally to this work