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DANIO RERIO (ZEBRAFISH): A COST EFFECTIVE ANIMAL MODEL FOR ANTI-TUBERCULOSIS DRUG RESEARCH

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Abstract

Animal models help us to understand and explore disease mechanisms. Every disease causing organism has its own evolving genome and interacting ways with the environment, which demands new animal models for studying them. Versatile and cost effective disease models are always in demand for drug discovery. Laboratory mouse and rat animal models, though well appreciated in the fields of drug discovery and basic science research is getting replaced with the disease models of Zebrafish as they are easy to breed, hold and discard. Cost effectiveness and the less implied Animal ethics regulations have made researchers in the West to shift to this model. Although Zebrafish models on various diseases are available, tuberculosis disease model is discussed here for the benefit of researchers interested or involved in anti-tuberculosis drug discovery.

Key Words: Antituberculosis, Drug discovery, Zebrafish, Animal model.

Introduction

Robert Koch, the celebrated German physician and microbiologist discovered the causative agent of tuberculosis, Mycobacterium tuberculosis in the year 1882.1 Since then, there is a severe competition among the drug discoverers in developing a drug that could hunt down this menace. The race is still on with the emergence of more complex and virulent MDR and XDR strains of the tubercle bacilli. Annual Tuberculosis report [(2015)] of WHO reports TB as one of the major health problem in the South East Asian region of the World with an estimated incidence of 3.4 million new cases of TB occurring each year. 2 India stands first among the 22 high TB burden countries in the World with 24% of the estimated global incidence and 20% of global TB related deaths. In this existing critical situation and with our research institutes joining the race in the field of drug discovery against this microbe, it becomes necessary to introduce to the early career researchers interested in anti-tuberculosis research with this easy to set-up, cost effective and feasible research unit in their respective work place. The disease model to be discussed, *Mycobacterium marinum* is not the direct tuberculosis animal model but a surrogate model that's genomically so close to that of the original.

Zebrafish- An introduction

Dania rerio (Zebrafish) is a tropical fresh water fish native to India, Bangladesh, Nepal and Burma. It belongs to the family Ciprinidae. Adult fishes grow to a size of 4cm in length with a lifespan of 4 years. They become fertile by 4-5 months after the hatch. They bear the name of zebra for the blue stripes they have on the side of the body. Sexual dimorphism exists and is easy to identify. They grow well with the commonly available aquarium food flakes and breed

easy in captivity. Thereby raising the fries and to convert the available lab space into a small animal breeding and holding unit is possible with the purchase of a few small aquarium tanks.

Danio rerio (Zebrafish)

AFB stain shows positive for M.marinum

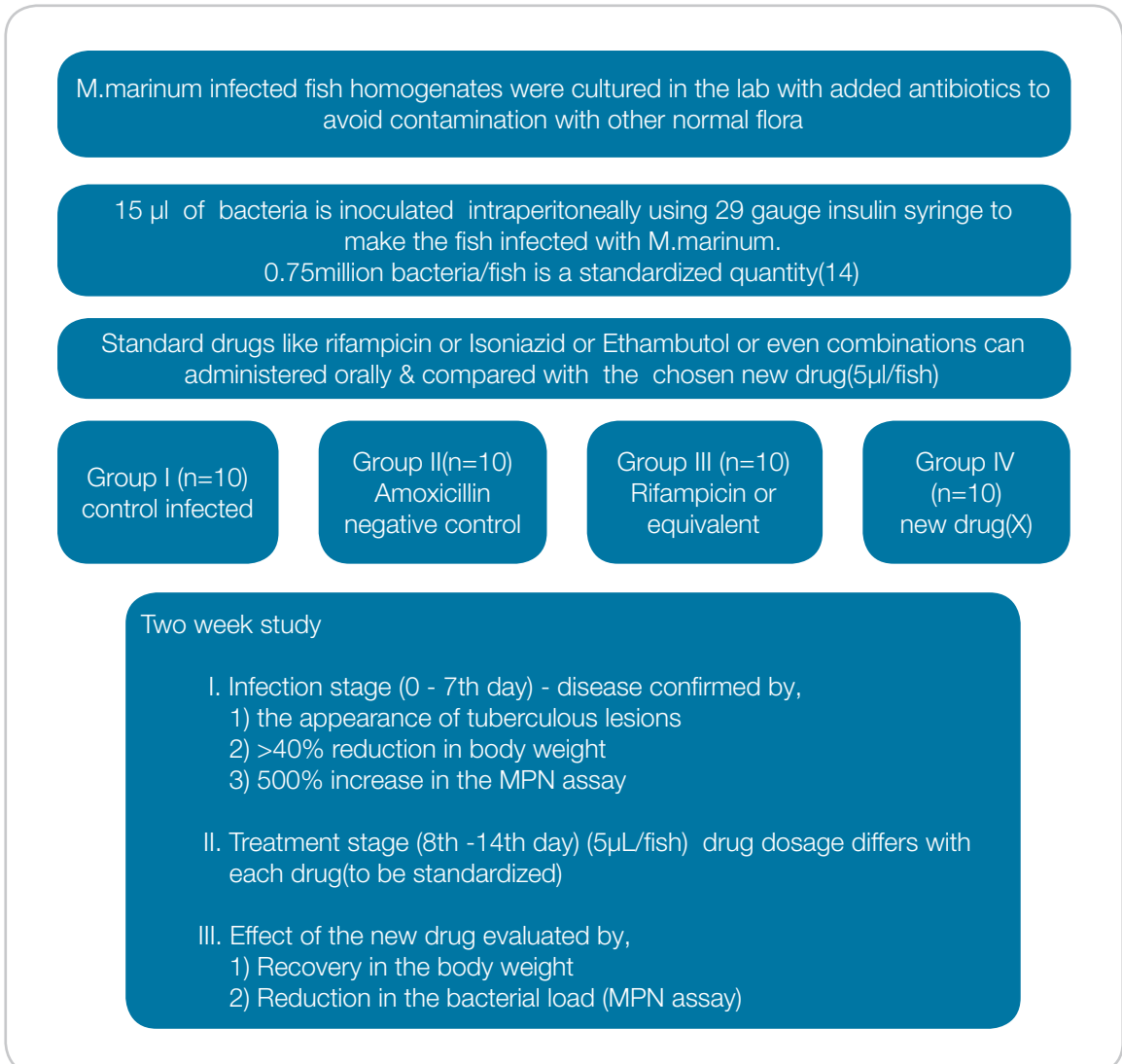
Zebrafish as a disease model

Dr. George Streisinger, in the early 1970's from the University of Oregon established Zebrafish as an animal model for genetic studies.[3] Later, his colleagues developed the embryonic developmental map for the same. Zebrafish, as a vertebrate has similarities to mammals at both the genetic & tissue levels thereby with the available standardized methodologies available for both transgenesis and gene knockdown techniques, they serve best animal models.[4] There are well established Zebrafish disease models for diseases like Parkinson's disease 5-7 Kidney function,[8] Porphyria, Ocular disorders, Cardiomyopathy, Muscular dystrophy, Ear defects, Alzheimer's disease, Melanoma, Pancreatic carcinoma and other Intestinal cancers.[9] Although the common mouse model has occupied a strong position in the scientific labs due to its mammalian physiology, experimental limitations are there to use them in large scale studies which requires space, manpower and money. Zebrafish has its own advantages with its small size, easy to breed and hold. Also the entire development of the embryo takes place outside the body and is highly transparent to visualize, intervene and document the changes or results. At least 70% of human protein coding genes including disease causing genes have ortholog in Zebrafish which suggests its importance for disease modeling.[10] With our understanding on the genetic basis of human diseases and with our expertise in deep sequencing strategies, there is a rise in the number of Zebrafish cancer disease models. Thus Zebrafish disease models are becoming an authentic alternative for mouse or rat models.

Tuberculosis disease model in Zebrafish

As discussed earlier, Tuberculosis is a deadly disease and the hunt for better drugs is happening across the globe. Tuberculosis disease burden in India is huge and devastating with the emergent of drug resistant tubercle bacilli. A surrogate model for tuberculosis on Zebrafish will serve the purpose for the preliminary screening of synthetic or herbal anti-tuberculosis drug formulations. In 1926, Mycobacterium marinum that causes fish tuberculosis was first isolated. M. marinum being a genetically close associate to Mycobacterium tuberculosis infects Zebrafish easily. Exposure to infected fish or to the aquarium water causes cutaneoous infections in humans.[11] This bacteria does not grow in temperature of more than 370C [12] and it has a relatively short generation time. Though M.marinum is associated to M.tuberculosis, it does not have the potency to affect humans, except in immunocompromised individuals causing a serious threat. However M.marinum can be handled safely in a lab using BSL – II precautions.[13]

The parameters for the effect of the new drug given orally in a disease model for drug discovery can be assessed by estimating change in the bacterial count and body weight. Methodology is validated with anti tuberculosis drugs such as rifampicin, isoniazid, and moxifloxacin.[14]



Multiple trials with different dosages of the same drug or with combinations of more than one drug can be experimented any number of times as the time period of the entire study is two weeks with <50 small fishes and more importantly within a less budget. Data collection and statistical analysis follows the same as for any other animal studies.

Further Implications

Every department can initiate research with this new animal model not only in terms of anti-tuberculosis drug research but also with other basic science research areas like behavioral, hematological, motor and sensory physiology. Institutions can have a well equipped single Zebrafish animal facility that can inbreed fishes and supply to the needy individual researcher. Literature review from Indian subcontinent showed much of the embryonic toxicological research in Zebrafish model than in any other available disease models. It is high time that medical colleges can adopt this model as a replacement for the Animal house facility due its cost effectiveness and less or no regulatory issues.

Conclusion

Zebrafish infected with Mycobacterium marinum is found to be a validated surrogate model for anti tuberculosis drug research. This cost effective model is gaining much importance and popular among researchers worldwide. It is high time that we need to use this model to discover a drug that could wipe out the tuberculosis menace well before it fails the mankind.

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