To Live or Not To Live: Liver Decides!

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Ever wondered how a lizard sheds its tail and regrows a new one later? How cutting an earthworm into two halves leads to formation of two new earthworms? Scientists call it 'Regeneration'. Can humans regenerate too?? Well, not completely. The closest that we humans could get; is regenerating our livers. Liver is one of the most hardworking and multifunctional organ in our body. It regulates key physiological processes like nutrient processing, xenobiotic detoxification, waste processing and excretion, energy and nutrient storage and regulation and production of serum proteins. The functions that it performs are so vital that any failure to perform them can lead to serious patho-physiological conditions and in extreme cases even death. The liver homeostasis, also termed as 'hepatostat', therefore; needs to be very critically maintained. Could this be why nature has conserved the regenerative ability of liver? May be.

So how exactly does liver dysfunction occur? In a normal scenario, liver bears all the metabolic load of the body while maintaining its own needs. The routine consumption of alcohol, coffee, drugs, heavy metals and energy drinks makes the load even worse. A long term exposure of drugs prescribed to treat various diseases e.g. tuberculosis, add to the stress of already strained liver; eventually causing liver damage. However, in most such situations the treatment cannot be terminated, or else, the patient will die of the disease. Drug induced liver injury (DILI) is one of the mostly cited reasons for withdrawal of an approved drug from the market and unfortunately, while there are many drugs that affect liver, there are no drugs available as such to reinforce liver

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functions. Currently, the only treatment that has been followed for severe liver diseases is liver transplantation. The surgical procedure is extremely complicated, risky and expensive. Also, the number of patients needing a liver transplant outnumbers the donors available. Therefore, there is a major emergency in the field that demands urgent attention towards finding therapeutics that could improve liver regeneration and/or protect from DILI.

Our research team led by Dr. Chetana Sachidanandan at CSIR-Institute of Genomics and Integrative Biology, New Delhi, has taken up this challenge. We utilise zebrafish as a model system to address this problem. As the mechanisms of liver function are very well conserved between humans and zebrafish, discoveries made in zebrafish can be extrapolated to humans. In addition, the small size and transparent nature of zebrafish embryos makes it an ideal system to visualise internal organs and carry out chemical screens for discovery of therapeutics. Using this handle, the team utilised a transgenic zebrafish line which has a red fluorescent protein (mCherry) in its liver that allows visualization of liver in live organism. The protein is fused with a bacterial enzyme Nitroreductase. When these transgenic fishes are treated with a drug Metronidazole (Mtz), it gets converted to a toxic product thereby killing liver cells. This way, liver damage can be achieved and as Mtz is washed off, liver starts regenerating. Both these processes can be monitored by changes in mCherry fluorescence. Since liver regeneration is directly dependent on the type and extent of damage, we needed to standardise these parameters. It has been established that liver can regenerate as much as 75% of hepatic insufficiency. Our team figured out 2.5mM Mtz treatment for 24 hours is sufficient to cause almost 70% reduction in liver size and also allow regeneration by 48 hours post Mtz removal. Using multiple means, we demonstrated the robustness of this model and established an assay to perform a chemical screen for hepatoprotective and/or hepatoregenerative drugs.

In order to perform a targeted chemical screen, we used hundreds of small molecules that could lead to potential drugs in future. 3 dpf (days post fertilization) embryos were treated with DMSO (control), Mtz and Mtz along with small molecule. After 24 hours of exposure, the embryos were scored for mCherry fluorescence as a marker of liver size. As expected, in comparison to control embryos, Mtz treated embryos showed liver damage. However, what our team looked for was; which compounds when co-treated with Mtz were potent enough to protect from liver damage caused by 'only Mtz' treatment. We were able to identify a handful of them, of which CS-KIH03 served to be the most potent and promising one.

As mentioned earlier, in spite of the associated liver injury, the prolonged drug treatment for many diseases still cannot be terminated. The best alternative to this is to have an antidote that would prevent the upcoming liver damage. In clinical terms, it is known as prophylactic treatment. Considering this need, the team tested if pre-treatment of CS-KIH03 could lower the risk of upcoming liver injury by Mtz. Interestingly, the small molecule showed a potent prophylactic activity when post treated with Mtz. We further tested if CS-KIH03 also possesses regenerative activity. After almost 70% of liver damage, the transgenic zebrafish embryos were allowed to regrow their liver naturally and in presence of the small molecule. We were delighted to find that the embryos treated with small molecule were able to show captivating increase in the rate of regeneration as compared to the naturally regenerating livers. This led to further characterization of CS-KIH03 as a therapeutic for DILI.

Clinically, the most common drug for liver injury is Acetaminophen (Paracetamol). Our team selected two more drugs called Isoniazid (drug for TB) and Thioacetamide that have been reported to cause liver injury. We created similar DILI models in zebrafish using all these drugs and characterised them. Further, we performed experiments to assess whether CS-KIH03 is able to protect from liver injury caused by all these drugs. To our surprise, the newly identified small molecule could successfully prevent liver injury in all three toxicological models of liver injury, highlighting its generic potential.

In all, our study utilises a genetic model for liver-ablation in zebrafish in order to perform a chemical screen to discover therapeutics for DILI. We found CS-KIH03 to be a highly potent compound with hepatoprotective, regenerative and prophylactic activity. In addition, protection by CS-KIH03 in various kinds of toxicological models of DILI highlighted the potential of this drug to be the prospective antidote against liver damage. Further, our team aims to dissect the mechanism of action of this drug using small molecules that are known to have similar functions like CS-KIH03. The mechanisms are under investigation and knowledge earned from this study would pave a way towards understanding DILI in more detail as well as discovering therapeutics of targeted functions.