Tales from the deep: reeling in renal failure

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A MAJOR FUNCTION OF THE KIDNEYS is the concentration and excretion of toxic metabolites and drugs. Toxin-mediated acute renal failure (ARF) causes severe morbidity and mortality that are appreciably higher in older and more debilitated patients. Surviving individuals face a significant risk of requiring long-term dialysis or a progressive decline in renal function (14). An episode of ARF may limit the future use of drugs such as aminoglycosides (gentamicin and others) and cisplatin, even when these drugs are integral to the treatment of disease.

The aminoglycoside antibiotics constitute ideal coverage for a variety of infectious disorders. Because of a combination of high efficacy and low cost, they are some of the most commonly prescribed antibiotics for gram-negative infections (14). The main dose-limiting toxicities of this class of antibiotics comprise both nephro- and ototoxicity (13, 14). Despite significant research into the pharmacokinetics of aminoglycosides and the widespread implementation of dosing normograms and serum level testing, renal toxicity still occurs in up to 50% of patients receiving these antibiotics. Of these nephrotoxic injuries, more than half occur after completion of therapy and recovery to normal renal function is often incomplete (14). Similarly, cisplatin chemotherapy has revolutionized the treatment of a wide variety of solid tumors and is still the standard treatment for curing particular tumor subtypes, most notably germ cell tumors (5). Nephrotoxicity is an important dose-limiting toxicity of cisplatin that is also seen in some patients receiving carboplatin and newer generations of platinum analogs (9). A better understanding of the mechanisms of nephrotoxicity of these classes of compounds through in vivo modeling may yield important insights for the development of rational algorithms or targeted therapies, to ameliorate the nephrotoxicity of these drugs.

In this issue of American Journal of Physiology-Renal Physiology, Hentschel et al. (10) describe a series of elegant experiments in which they demonstrate the utility of the zebrafish to both model the nephrotoxic effects of gentamicin and cisplatin and to be used as a platform for screening therapeutics that may ameliorate these toxicities. The zebrafish, Danio rerio, is a superb genetic system for physiological and developmental studies and for translating basic biological insights into understanding human pathology. Zebrafish are small (3- to 4-cm adults) and easy to raise, with a short generation time of 3 mo. Because their developing embryos are transparent, embryonic organ formation is easily studied under a dissecting microscope. Zebrafish embryos develop rapidly, with a beating heart and visible vasculature and hematopoietic system by 24 h. By 4 days postfertilization, the kidney and complete gastrointestinal tract, including the liver, gallbladder, pancreas, and intestines, are functional. Zebrafish females can lay hundreds of eggs at weekly intervals. The organism maintains the diploid state, which makes analysis easier, in contrast to other fish that can be tri- or tetraploid (1, 16).

The mesonephric zebrafish kidney demonstrates structural components similar to the metanephric mammalian kidney, although lacking collecting and complex nephron systems. At 3–4 days postfertilization, the age at which the current studies were undertaken, the zebrafish embryonic kidney consists of a glomerulus and a pair of nephrons (3). Despite this simplicity, the developing kidney is composed of cell types similar to those seen in the mammalian kidney including podocytes, and fenestrated epithelia, and functions to clear fluid and waste products (4).

Aminoglycosides penetrate epithelial cells poorly; thus the proximal tubule cells of the kidney must actively concentrate them. Acidic phospholipids and the endocytic receptor megalin have been implicated in aminoglycoside uptake (8, 12). Targeted disruption of megalin in the mouse ameliorates aminoglycoside toxicity (15). Of note, results of the zebrafish genome initiative (http://www.ensembl.org/Multi/blastview?species=Danio_rerio) demonstrate a possible orthologous megalin gene on the telomere of zebrafish chromosome 10. In humans and currently used model organisms, renal aminoglycoside toxicity is marked by glomerular and tubular damage, phospholipiduria, and lysosomal phospholipidosis (7). Hentschel et al. (10) describe a similar pathology in the zebrafish after aminoglycoside injection. Cisplatin toxicity in proximal tubular cells is characterized by lysosomal size alteration and mitochondrial vacuolization, but the primary effects are likely an inhibition of protein synthesis, reduced glutathione (GSH), and protein-GSH depletion (11). The present studies demonstrate a similar pattern of nephrotoxicity in the zebrafish kidney after the administration of cisplatin. These similar pathological changes in the zebrafish are accompanied by a decrease in the glomerular filtration rate (GFR) after gentamicin and cisplatin injection. Thus, not only does the zebrafish pronephros replicate the injury seen in human nephrotoxicity with these agents, but a corresponding functional loss is seen as well. Because the zebrafish model system replicates both functional and pathological changes seen in human aminoglycoside and cisplatin nephrotoxicity, these data support the hypothesis that renal physiology is conserved between zebrafish and humans, at least as it pertains to toxin-mediated ARF.

The authors’ demonstration that the zebrafish model system may be used as a tool for screening potential nephrotoxic mitigating agents is perhaps the most exciting part of the present studies. Previously, taurine has been shown to reduce gentamicin nephrotoxicity in other model systems (6), and the Omi/HtrA2 inhibitor Ucf-101 has been shown to ameliorate cisplatin toxicity in both in vitro and in vivo studies (2). Because the authors show that these agents function similarly in the zebrafish, the zebrafish model not only becomes a

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platform for investigating ARF, but it is also a tool to rapidly screen for new compounds that mitigate aminoglycoside or cisplatin nephrotoxicity. A significant advantage of the current studies over other in vivo model systems is the authors’ use of a novel fluorescent technique to determine GFR directly in zebrafish embryos. This innovative approach involves injecting fluorescently labeled dextran or inulin into the transparent zebrafish embryo, permitting direct calculation of GFR in the whole organism. The system takes advantage of the optical clarity of the zebrafish embryo, facilitating the rapid quantification and potential automation of such screens.

The ability to define developmental and regenerative pathways in the zebrafish using reverse genetic, forward genetic, and chemical screens has had a profound impact on our knowledge of human disease pathways and treatment. The experiments reported by Hentschel et al. (10) in this issue of American Journal of Physiology-Renal Physiology support the addition of the zebrafish as an important new model organism for understanding the pathophysiology and treatment of ARF. Clearly, the strengths of the zebrafish in visualization and screening complement the advantages of the existing ARF model systems, such as targeted gene inactivation in the mouse. It will be exciting to see how insights derived from these and other similar studies will eventually lead to a better understanding of ARF and improved therapies for its treatment.

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GRANTS

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REFERENCES