A Fish Model of Renal Regeneration and Development

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Abstract

The fish kidney provides a unique model for investigating renal injury, repair, and development. Like mammalian kidneys, fish kidneys have the remarkable ability to repair injured nephrons, designated renal regeneration. This response is marked by a recovery from acute renal failure by replacing the injured cells with new epithelial cells, restoring tubule integrity. In addition, fish have the ability to respond to renal injury by de novo nephron neogenesis. This response occurs in multiple fish species including goldfish, zebrafish, catfish, trout, tilapia, and the aglomerular toadfish. New nephrons develop in the weeks after the initial injury. This nephrogenic response can be induced in adult fish, providing a more abundant source of developing renal tissue compared with fetal mammalian kidneys. Investigating the roles played by different parts of the nephron during development and repair can be facilitated using fish models with differing renal anatomy, such as aglomerular fish. The fish nephron neogenesis model may also help to identify novel genes involved in nephrogenesis, information that could eventually be used to develop alternative renal replacement therapies.

Key Words: development; fish; kidney; model; nephron; regeneration; repair

Introduction

The mammalian kidney's ability to repair sublethal toxic injury has been known for more than 100 yr (Podwyssozki 1885). Numerous nephrotoxicants have been used to demonstrate the pattern of cellular repopulation along the proximal tubule (Cuppage and Tate 1967; Oliver 1915; Reimschuessel et al. 1990b, 1991). Although the time course may vary, the basic pattern of regeneration after exposure to these nephrotoxicants is similar. The denuded basement membrane is lined by basophilic, flattened, squamous cells several days after the administration of the toxicant. Later, these cells develop into a cuboidal basophilic epithelium and eventually differentiate into a mature epithelium. This type of repair has been designated classically as **renal regeneration**, with regeneration referring to the repopulation of the existing nephron after cells have been destroyed. If, however, there is overwhelming injury to the nephron, including destruction of the basement membrane, the nephron will degenerate and the glomerulus undergoes fibrosis.

Another well-characterized response of the mammalian kidney is **compensatory renal hypertrophy**, or the enlargement of the remaining kidney after unilateral nephrectomy (Fine 1986; Kaufman et al. 1975). Unilateral nephrectomy in the neonate induces cellular multiplication in the remaining kidney (Karp et al. 1971; Sands et al. 1979) whereas in the adult, 80% of the increase in renal size is due to cellular hypertrophy (Johnson and Vera Roman 1966).

A third renal repair response, **nephron neogenesis**, has been described in fish (Reimschuessel and Gonzales 1998; Reimschuessel and Williams 1995; Reimschuessel et al. 1990a, 1993). Regeneration in mammalian kidneys in response to toxic injury does not include the development of new nephrons. Neither compensatory renal hypertrophy following unilateral nephrectomy, nor regeneration in mammalian kidneys in response to toxic injury, results in the development of new nephrons (Fine 1986; Kaufman et al. 1975; Kazimierczak 1982; Larsson et al. 1980). Postnatal development of new nephrons in mammals occurs only in the neonatal period of some species. For example, in the normal neonatal rat, the kidney continues to produce new nephron anlages for up to 3 days after birth (Larsson 1975, 1982; Neiss and Klehn 1981; Reeves et al. 1980). These anlages develop into mature nephrons by 12 days. After this period, however, no further nephron development takes place.

Although new nephron development occurs only in the neonatal period of some mammals (e.g., the rat), it proceeds throughout life in fish (Yasutake and Wales 1983). Intensely basophilic, compact developing tubules can occasionally be seen in normal adult fish kidneys. They are seen with greater frequency in young, rapidly growing fish. After nephrotoxicant-induced injury, however, the number of developing nephrons in both young and adult fish increases significantly (Brown and Reimschuessel 1998; Reimschuessel et al. 1990a, 1993). Because adult fish can respond to injury by nephron neogenesis, providing much more tissue than can be found in larval or embryonic kidneys, fish are excellent models for studying both renal regeneration and nephron development.

The following sections will examine more fully the normal development of the mammalian and fish kidney, the regenerative response in the mammalian and fish kidney, and finally the nephron neogenic response in fish.

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Development of the Vertebrate Kidney

Vertebrate development generally proceeds in the direction of anterior to posterior. The development of the kidney also follows this pattern. Anterior segments develop and become functional earlier than posterior segments, producing in sequence the pronephros, the mesonephros, and the metanephros (Berrill and Karp 1976; Fraser 1950; Goodrich 1958; Kerr 1919). The pronephros develops from the intermediate mesoderm located between the somatic mesoderm (myotome) and the lateral-plate mesoderm. Pronephric tubules develop in each segment from a solid mass of cells, the nephrotome (also called renal anlage or nephrogenic blastema).

The pronephros is functional throughout life in some genera of fish (*Fierasfer, Zoarces, Lepadogaster*) (Hickman and Trump 1969; Kerr 1919; Lagler et al. 1977). In most vertebrates, however, the pronephros degenerates and disappears as the mesonephros develops. As with development, this process progresses from the anterior to the posterior end.

The mesonephros arises from the nephrotome in more posterior segments. The tubular outgrowths, Bowman's capsule and glomerulus, form in a manner similar to that of the pronephros (Fraser 1950; Goodrich 1958; Kerr 1919). The nephrotome consists of a solid mass of cells near the pronephric excretory duct. A cavity forms in the center of this mass and forms the renal vesicle (Figure 1). As it grows, the vesicle is first C-shaped but soon develops an S-shape. The medial end of the S invaginates further and develops into the glomerulus with an outgrowth forming the peritoneal funnel. The remainder of the S becomes the primary tubule. The tubule grows out and fuses with the pronephric excretory duct, which eventually becomes the mesonephric or Wolffian duct. Secondary and tertiary tubules develop from nephrogenic blastema of the nephrotome, elongate, and fuse with the primary tubule, forming a collecting tubule. The tertiary tubules open into the secondary tubules. The tubules all elongate, coil, and intertwine, producing the mesonephros.

In the teleosts, the tubules of the anterior region of the mesonephros degenerate, and the "head kidney" becomes a hematopoietic, lymphoid, and endocrine organ. The mesonephros is the functional kidney of adult teleosts, and it is also functional in the mammalian embryo (Altschule 1930; Bremer 1916; Davies and Routh 1957; de Martino and Zamboni 1966; Fraser 1920; Leeson and Baxter 1957; Lewis 1920; MacCallum 1902; Tiedemann 1976).

The metanephros develops in the Amniota: reptiles, birds, and mammals (Berrill and Karp 1976; Fraser 1950; Goodrich 1958; Huber 1905; Kerr 1919). A ureteric bud develops as a diverticulum of the dorsal side of the mesonephric duct, and it grows forward and dorsally toward the remaining nephrogenic blastema. Except for the development of the loop of Henle, the development of the metanephric nephron parallels that of the mesonephric nephron: formation of the renal vesicle, the S-stage, fusion with the collecting duct, and elongation of the tubular portion.

In both the mesonephros and the metanephros, the nephrogenic blastema develops in close association with the Wolffian duct or the ureteric bud, respectively. In vitro experiments (Erickson 1968; Grobstein 1955, 1957) have shown that when the metanephric blastema is cultured together with ureteric bud, it undergoes tubulogenesis. Cell-to-cell contact is required for induction of tubule formation (Sariola et al. 1989; Weller et al. 1991). Branching of the ureteric bud is also dependent on co-culture with a metanephric blastema that has not yet undergone tubulogenesis (Humes et al. 1996; Sakurai and Nigam 1998). This blastema also simulates what occurs in vivo. The ureteric bud branches after entering the nephrogenic blastema, which in turn forms caps and differentiates into nephrons. Thus, there are complex interactions between the ductular and tubular elements during development of both the mesonephros and the metanephros.

Anatomy of the Teleost Kidney

The kidneys of fish are retroperitoneal, as in the mammal. The gross anatomy of the kidney varies in different species from distinctly bilobed cranial and caudal kidneys, to kidneys that are fused and intimately embedded between the vertebrae (Ogawa 1962). The cranial or "head" kidney contains hematopoietic, lymphoid, and endocrine tissue. The caudal kidney is composed of nephrons surrounded by hematopoietic and lymphoid tissue dispersed throughout the organ. Species variations in tubular segmentation also exist (Edwards and Schnitter 1933; Endo and Kimura 1982; Hentschel and Elger 1988; Hickman and Trump 1969; Longley and Fisher

Figure 1 General development of the mesonephric tubule. A solid mass of cells near the archinephric duct develops a cavity and forms the renal vesicle. As it grows, the vesicle is first C-shaped and then S-shaped. One end of the S-shaped stage indents further, forming the glomerulus, while the other end grows outward and fuses with the archinephric duct. The tubules elongate, coil, and intertwine as the nephron develops. RV, renal vesicle; C, C-shaped stage; S, S-shaped stage; DN, developing nephron.

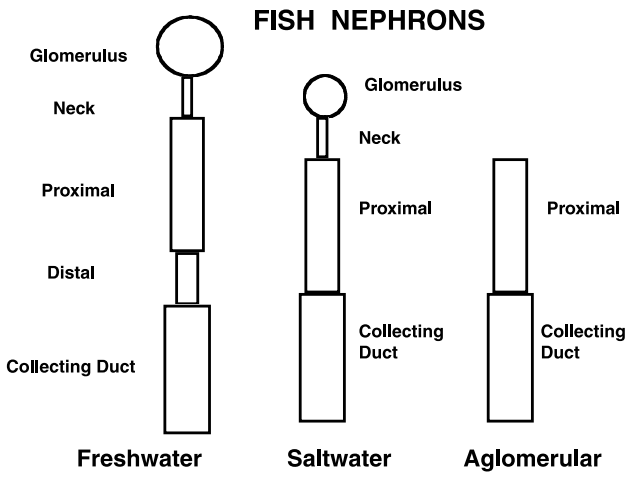

Figure 2 Schematic of different types of fish nephrons.

1954; Ogawa 1962; Sakai 1985) (Figure 2). These variations are most evident when comparing freshwater and marine species, which is not surprising because their environments make different demands on their kidneys. The freshwater nephron typically has a well-vascularized glomerulus, a ciliated neck segment, two proximal segments, a ciliated intermediate segment, a distal segment, and a collecting duct system. The mesangium of many marine forms is thickened, and the glomerular filtration rate is lower than that of freshwater fish. Some estuarine or marine fish have no glomeruli, and many marine teleosts have no distal tubule. These changes probably occurred as evolutionary adaptations when freshwater teleosts reinvaded the oceans (Hickman and Trump 1969; Smith 1939). Such differing anatomical types provide the creative research scientist unique opportunities to study aspects of renal function and development that would be impossible to study in mammalian models.

Renal Regeneration in the Mammalian Kidney

Renal regeneration of cells in an existing nephron has been well documented in mammals. Oliver (1915) described renal epithelial regeneration after administration of uranium nitrate in the rat, rabbit, and guinea pig. Since that time, there have been numerous reports of proximal tubular regeneration in the rat after ischemia or the administration of nephrotoxicants (Boti et al. 1982; Cuppage and Tate 1967; Haagsma and Pound 1980; Houghton et al. 1976; Ishmael et al. 1982; Kociba et al. 1977; Ormos and Gohus 1979; Peterson and Carone 1979; Spangler et al. 1980; Venkatachalam et al. 1981; Wellwood et al. 1976). The basic pattern of regeneration is similar for these nephrotoxicants. For example, in the rat, a sublethal dose of mercuric chloride causes the epithelium of the mid- and terminal portions of the proximal tubule to degenerate and eventually slough into the lumen of the tubule, leaving the denuded basement membrane intact. Within 3 to 4 days after the injury, the tubules are lined by basophilic, flattened squamous cells. Mitotic figures are often noted in these regenerating cells. By 5 to 7 days, the epithelium is composed of short, cuboidal basophilic cells. Most tubules regain normal morphology between 3 and 4 wk (Boti et al. 1982; Cuppage et al. 1972). This entire process of regeneration, which involves epithelial cell dedifferentiation, movement, proliferation, and redifferentiation, restores the functional integrity of the nephron (Abbate et al. 1999; Imgrund et al. 1999; Safirstein 1999). Renal function, as measured by creatinine clearance, parallels the structural changes after mercuric chloride toxicosis. Creatinine clearance is impaired during the first 5 days but improves during the latter phases (days 7-10) of regeneration (Cuppage and Tate 1967).

The regenerative response in rat proximal tubules after mercuric chloride administration is similar to that seen after hexachlorobutadiene (HCBD[1]) exposure. HCBD nephrotoxicity has been studied extensively in the rat (Berndt and Mehendale 1979; Davis et al. 1980; Gage 1970; Harleman and Seinen 1979; Ishmael et al. 1982; Kociba et al. 1977; Lock and Ishmael 1979). Unlike mercuric chloride, HCBD does not cause hepatocellular damage in addition to its effects on the kidney (Davis et al. 1980). HCBD causes marked necrosis and vacuolation in the proximal tubules. By 5 days, most of the injured tubules are lined by a cuboidal basophilic epithelium that contains many mitotic figures.

Renal function as measured by plasma urea increases 16 hr after HCBD injection, peaks at 3 days, and returns to normal by day 7 (Davis et al. 1980; Ishmael et al. 1982). The time course of the decline in function and subsequent improved function parallels the histopathological changes. In both mercuric chloride and HCBD nephrotoxicity, the appearance of a regenerating epithelium is followed by improved renal function.

Renal Injury and Regeneration in the Fish

Although renal injury has been well described in many fish species, the repair responses of the fish kidney have only recently been characterized in several fish species (Augusto et al. 1996; Reimschuessel et al. 1989, 1990b, 1996). Repair of the existing nephron after toxicant-induced injury occurs in goldfish, catfish, trout, zebrafish, and tilapia (Augusto et al. 1996; Reimschuessel and Biggs 1996; Reimschuessel et al. 1990b, 1993). This process is similar to one that occurs in the mammalian kidney. There is an initial phase of cell death and denuding of the basement membrane (Plate 1A, page 305). During the ensuing days, a flattened basophilic epithelium repopulates the remaining denuded basement membrane (Plate 1B, page 305). This process is accomplished by cell

[1]Abbreviations used in this article: HCBD, hexachlorobutadiene; IGF-I, insulin-like growth factor-I; WT-1, Wilms' tumor transcription factor gene.

migration and replication both proximal and distal to the lesion (Reimschuessel et al. 1990b). Although the function of the injured fish kidney is difficult to assess, clinical signs such as exophthalmia, ascites and low hematocrit, and hypoproteinemia due to osmoregulatory failure are observed during the acute injury phase (Plate 2, page 306). As the nephrons regenerate, the ascites resolves and hematological parameters return to normal (Reimschuessel et al. 1989). These findings correlate well with what has been found in mammalian models of renal repair.

Nephron Neogenesis in the Fish

The nephrotoxic injury phase in fish is first followed by the repair phase. There is, however, an additional phase that makes the fish kidney valuable as a model for renal repair and regeneration. The fish kidney exhibits a unique nephron neogenic response with de novo nephron development (Augusto et al. 1996; Reimschuessel et al. 1990a, 1991, 1993). A similar neogenic response is not observed in mammals.

The nephron neogenic response has been described in multiple fish species, including goldfish (Reimschuessel et al. 1990a), rainbow trout (Reimschuessel et al. 1993), tom cod and catfish (Cormier et al. 1995), zebrafish (Reimschuessel and Biggs 1996), tilapia (Augusto et al. 1996), and even the aglomerular toadfish (Brown and Reimschuessel 1998). A variety of toxicants, with differing mechanisms of toxicity, have been used to demonstrate this response. These toxicants include HCBD (Reimschuessel et al. 1990a), mercuric chloride (Reimschuessel and Gonzales 1998), tetrachlorethylene (Reimschuessel et al. 1993), and gentamicin (Brown and Reimschuessel 1998; Reimschuessel and Williams 1995). The new nephrons that form in goldfish after nephrotoxicant-induced injury follow the same pattern of development as is observed during nephrogenesis in developing mammalian kidneys. Specifically, basophilic clusters of cells adjacent to collecting ducts form renal vesicles and S-shaped tubules, and the tubular outgrowths then fuse with the collecting ducts. Glomerular development results in glomeruli with vascular tufts, parietal and visceral epithelia, and a clear Bowman's space (Plate 3, page 309). The nephrons develop over a period of 2 to 4 wk after exposure to the toxicant. This neogenic response thus provides a unique model for studying developing nephrons in an adult vertebrate organism.

Nephron Neogenesis in Aglomerular Fish

The anatomical variations in kidney structure found in various fish species provide a unique opportunity to study structure-specific development and function. Specifically, whereas freshwater fish species have nephrons composed of a glomerulus, a neck segment, proximal tubules, distal tubules, and a collecting duct system, differing from mammalian kidneys only in the absence of the loop of Henle and presence of interstitial myelopoietic tissue, the estuarine toadfish is aglomerular (Hickman and Trump 1969). Toadfish, with nephrons that lack a glomerulus and distal tubules, thus represent a naturally occurring knockout model of glomerular structure and function. Toadfish have historically been used in studies that have had profound implications for understanding mammalian biology and physiology. For example, studies in the aglomerular toadfish provided the first definitive evidence for the role of renal tubular secretion in the excretion of xenobiotics (Marshall and Grafflin 1928). Such studies were not possible in mammalian systems due to the potential contribution of the glomerulus.

Toadfish have been shown to be extremely sensitive to gentamicin nephrotoxicosis at doses that are therapeutic for other fish species (Reimschuessel et al. 1996). There are also major differences in the pharmacokinetics of gentamicin in goldfish and aglomerular toadfish (Jones et al. 1997) (Figure 3). These differences are due to the fact that gentamicin is excreted primarily via glomerular filtration. Because toadfish are aglomerular, serum levels of gentamicin remain high for several weeks after treatment. However, as soon as the serum levels of gentamicin are below detectable levels, toadfish kidneys also produce new nephrons (Brown and Reimschuessel 1998) (Plate 4, page 308). It is possible that future studies using aglomerular toadfish will identify genes involved in glomerulus-specific development and repair. Because approximately 25% of all cases of pediatric chronic renal disease involve glomerular dysfunction (Watson 1996), the identification of genes involved in normal glomerular development and repair could have important implications for the development of therapies for glomerular diseases.

Molecular Events in Renal Development, Repair, and Disease

The precise regulation of cell proliferation and cell death is critical for proper kidney development, repair, and homeo-

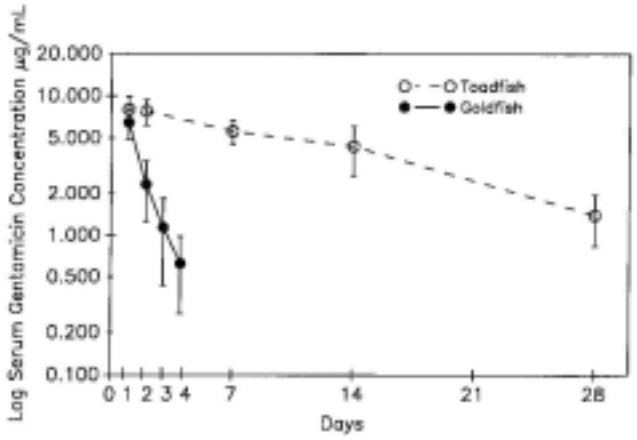

Figure 3 Mean gentamicin concentrations ((g/mL serum) in goldfish and toadfish after administration of 3.5 mg of gentamicin/kg body weight.

stasis in the mature organ. Accordingly, gene products that function to initiate cell division, maintain cell cycle progression, and terminate proliferation via growth arrest or cell death play essential roles in this dynamic process. Many genes are expressed both during development and during regeneration, indicating that some events in renal regeneration recapitulate renal development. For example, *myc* (Bendit et al. 1991; Cowley et al. 1989; Mugrauer and Ekblom 1991), *Pax-2* (Imgrund et al. 1999), and insulin-like growth factor-I (IGF-I[1]) (Matejka 1998) have been identified in developing kidneys and in repairing injured kidneys.

In addition to gene products with global functions in cell proliferation (e.g., immediate early genes such as *c-myc*) or cell death (e.g., *bc1-2*), studies in transgenic and knockout mice have identified several genes that exhibit functions restricted to the kidney and related tissues. For example, mice carrying a targeted mutation of the Wilms' tumor transcription factor gene (WT-1[1]) die in utero due to a failure in kidney and gonad development (Kreidberg et al. 1993). These mice do not express *Pax-2*, a putative regulatory factor normally expressed in early kidney development (Dressler and Drouglas 1992; Dressler et al. 1993). Because *Pax-2* transgenic mice also exhibit aborted kidney development (Torres et al. 1995), the regulation of *Pax-2* is thought to be an important function of WT-1 in kidney development. Abnormal kidney development has also been reported in *c-ret* knockout mice (Schuchardt et al. 1994), whereas overexpression of the *Hox* gene, which is implicated in normal renal development (Cillo et al. 1992; Clapp and Abrahamson 1993; Redline et al. 1994), results in the development of renal cancers. Thus, although several gene products required for kidney development have been identified, it is still necessary to isolate upstream regulators and downstream effectors of these genes to understand the molecular events in renal development more completely.

Gene products that function in activities essential to the viability of the organism, such as development, stress response, and regulation of cell proliferation, are highly conserved among vertebrates. Indeed, fish homologs of c-*myc* (Schreiber-Agus et al. 1993; Van Beneden et al.1986; Zhang et al. 1995), *c-ret* (Marcos-Gutierrez et al. 1997), *Hox* (Levine and Schechter 1993; Ruddle et al. 1999), IGF-I (Perrot et al. 1999), and WT-1 (Kent et al. 1995) have been identified. The nephroneogenic repair in fish and renal development in mammals proceed through similar stages, suggesting that conserved gene products mediate these similar molecular events.

The fish nephron neogensis model system may help to identify novel genes involved in nephrogenesis (Liu et al. 1998). Identification of these genes represents a requisite first step toward understanding the genetic mechanisms involved in nephrogenesis and the molecular basis for the restricted nephrogenic response observed in mammals. Ultimately, this information could be used in the development of alternative renal replacement therapies based on the induction of de novo nephrogenesis in diseased kidneys.

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