Gentamicin-induced nephrotoxicity and nephroneogenesis in *Oreochromis nilotica*, a tilapia fish

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ABSTRACT: The renal response to 2 doses of gentamicin (low dose of 5 mg kg⁻¹ of body weight and high dose of 25 mg kg⁻¹ of body weight) was examined in the tilapia fish *Oreochromis nilotica*. Gentamicin exposure induced acute tubular necrosis that peaked in severity at 2 d following intraperitoneal injection of the high dose and at 4 to 7 d following injection of the low dose. Necrosis following high dose exposure was more severe than that following low dose exposure. Histochemical staining for gamma glutamyl transpeptidase showed the site of injury to be localized in the proximal tubules. Immunohistochemical staining with 5-bromo-2'-deoxyuridine was used to mark cell proliferation during regeneration and nephroneogenesis. Regeneration of epithelial cells along the basement membrane of damaged tubules and development of new nephrons were both documented following nephrotoxic injury. Although the overall pattern of renal response to gentamicin exposure was similar to that which has been previously documented in other freshwater fish species, the commercially important tilapia fish was significantly more sensitive than other fishes.

KEY WORDS: Development · Fish · Gentamicin · Kidney · *Oreochromis nilotica* · Nephroneogenesis · Nephrotoxicity · Regeneration · Tilapia

INTRODUCTION

Gentamicin is an aminoglycoside antibacterial agent that inhibits bacterial protein synthesis. This drug has a broad spectrum of activity against Gram-negative bacteria. The severe nephrotoxicity of gentamicin at therapeutic doses is, however, a major impediment to routine therapeutic use of this agent (Houghton et al. 1976, Kaloyanides & Pastoriza-Munoz 1980, Porter & Bennett 1981, Seale & Rennert 1982, Silverblatt 1982, Cojocel et al. 1984).

The study of the bacterial pathogens of fish and their sensitivity to antimicrobial agents, including gentamicin, has expanded rapidly (Ceschia et al. 1987, Schmitt et al. 1989, Fryer et al. 1990, Baya et al. 1991, Borrego et al. 1991), and the use of aminoglycosides

in the treatment of fish bacterial infections is well described (Stoskopf et al. 1986, Stoskopf 1988). The pharmacokinetics of gentamicin has been evaluated in the channel catfish (Rolf & Setser 1984). As clinical use of gentamicin in fish has increased, so has documentation of its toxicity in fish species, specifically its nephrotoxicity (Chamie & Reimschuessel 1994, Reimschuessel & Williams 1994, Rokokous et al. 1994). Previous work described the histopathological changes in the fish kidney following exposure to gentamicin (Reimschuessel & Williams 1994, Rokokous et al. 1994). Study of gentamicin nephrotoxicity in goldfish *Carassius auratus* has shown characteristic changes in the proximal tubules of the nephron, including nuclear pyknosis and sloughing of the tubular epithelium (Reimschuessel & Williams 1994). The most startling finding to have arisen from these studies is the remarkable reparative ability of kidney tissue in the investigated fish species.

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The ability of fish to regenerate apparently normal tissues and organs following injury or removal has long been known (Oliver 1915, Goss 1969). The regeneration of fish tissues following nephrotoxic insult has recently been the focus of much investigation (Reimschuessel et al. 1990, 1993, Lombarte et al. 1993, Chamie & Reimschuessel 1994, Reimschuessel & Williams 1994, Rokokous et al. 1994). Immunohistochemical staining for the uptake of 5-bromo-2'-deoxyuridine (BrDU) has been used to detect cellular replication (Gratzner 1982, de Fazio et al. 1987, Schutte et al. 1987) and document the patterns by which fish kidneys regenerate. Goldfish *Carassius auratus* exposed to nephrotoxic injury repaired kidney tissue by 2 distinct mechanisms. The first of these mechanisms is shared with mammals and involves repopulation of denuded tubules by replication and migration of tubular epithelial cells along the intact basement membrane. The second mechanism is unknown in mammals and involves nephroneogenesis, the creation of new nephrons, in their entirety, from small clusters of basophilic-staining cells in the renal interstitium (Reimschuessel et al. 1990). Nephreneogenesis following sublethal toxic insults may have profound implications for reversing serious renal injury resulting from aminoglycoside antibacterial therapy in fish. If nephreneogenesis occurs in other fish species, and not merely goldfish and salmonids, indications for the use of aminoglycosides in the treatment of bacterial fish diseases may be expanded.

To date, no studies have been reported on the nephrotoxicity of gentamicin in the tilapia fishes, or on the capacity of these species for nephreneogenesis. *Oreochromis nilotica*, an African cichlid fish, is extensively cultured and harvested as a food source. The hardiness, ready availability, and commercial importance of this fish make it exceptionally well suited for investigation as a representative of the tilapia fishes.

METHODS

Expt 1: gentamicin exposure. A total of 270 juvenile tilapia *Oreochromis nilotica*, measuring 3 to 8 cm in length and weighing 5 to 20 g, were arbitrarily divided into 3 groups: control, low dose, and high dose. Control fish received a single intraperitoneal (IP) injection of 10 ml kg⁻¹ sterile 0.09% saline solution. Low dose fish received a single IP injection of 5 mg kg⁻¹ gentamicin as 10 ml kg⁻¹ of a 0.5 mg ml⁻¹ solution of gentamicin in sterile saline solution. High dose fish received a single IP injection of 25 mg kg⁻¹ gentamicin as 10 ml kg⁻¹ of a 2.5 mg ml⁻¹ solution of gentamicin in sterile saline solution. Fish were maintained in filtered tap water at 70 to 74°F (21–24°C) in established 20 gallon (75 l)

aquaria with a stocking density of 30 or fewer fish per aquarium. Fish were fed a ground commercial fish diet (Zeigler Brothers, Gardner, PA, USA). Water quality was monitored with weekly assessments of ammonia and nitrite and maintained with 50% water changes weekly. Mortality of fish following injection was monitored and recorded daily.

Five fish from each group were sampled for histologic examination at 0.25, 0.5, 1, 2, 3, 4, 7, 14, 21, 28, 35, and 42 d following injection. Fish were euthanized by anesthetic overdose with tricaine methane sulfonate (MS-222, Sigma Chemical Co., St. Louis, MO, USA) followed by cervical dislocation. Kidney tissue was fixed *in situ* by immersion in a phosphate buffered 4% formalin/1% glutaraldehyde solution (McDowell & Trump 1976). Fixed kidneys and surrounding tissues were then routinely processed for paraffin sectioning. Sections (6 µm) were cut and stained with hematoxylin and eosin (H&E). All sections were encoded and randomized to prevent bias in interpretation and then examined by light microscopy. Pathological changes (interstitial edema, tubular dilation, necrosis, and epithelial regeneration along the tubule) were independently graded on a scale ranging from 0 to 4 indicating increasing degree and severity. Scores from the 2 gentamicin-treated fish groups were then compared with each other and with control fish for each time period. Significant differences between groups were determined by *t*-test analysis (2-tailed, 2-sample of unequal variance, Microsoft Excel version 4.0).

Nephreneogenesis was quantified by counting the number of developing nephrons found per unit area of caudal kidney tissue section on the encoded tissue slides. Developing nephrons were identified by their intense basophilia in comparison to surrounding tissue and their relatively smaller size and dense conformation (Reimschuessel et al. 1990, 1993, Reimschuessel & Williams 1994). The resulting ratio was compared across all groups from each time period. Statistical differences between and within groups and time periods were distinguished as described above.

Expt 2: staining for GGT activity. The enzyme marker gamma glutamyl transpeptidase (GGT) was used to identify the proximal tubules and to document the presence of normal enzyme activity in the fresh, frozen tilapia kidney. Two juvenile tilapia with weights of 5 and 7 g and lengths of 6.5 and 7.7 cm, respectively, were utilized. The control fish received a single IP injection of 10 ml kg⁻¹ sterile water. The treated fish received a single IP injection of 10 ml kg⁻¹ of a 2.5 mg ml⁻¹ solution (25 mg kg⁻¹) of gentamicin in sterile water. Food was withheld following injection, and fish were maintained in isolated 50 gallon (190 l) aquaria with flow-through systems of filtered tap water.

Both fish were euthanized 3 d following injection. The caudal kidneys were removed and divided into 2 pieces. The first piece was fixed in 10% formalin and routinely embedded in paraffin and processed for H&E sectioning. The second piece was embedded in Tissue-Tek II O.C.T. Compound® (Miles, Inc., Elkhart, IN, USA), fast frozen with Frostbite® (Surgipath, Richmond, IL, USA), and sectioned to make 6 µm frozen section slides.

Earlier investigation had demonstrated that the established protocol utilizing formalin fixation resulted in loss of staining for GGT activity in tilapia renal tissue. To determine a method for obtaining an optimal combination of tissue fixation and staining for enzyme activity, frozen sections received different treatments. One frozen section slide received no post-fixation. The other frozen slides were post-fixed with a 1 min wash of chilled 95% ethanol, acetone, or 10% formalin. Frozen slides were then rinsed in phosphate buffered saline (PBS) and stained for GGT activity following established protocol (Rutenburg et al. 1969). Following GGT staining, 1 section on each slide was counterstained with Gills hematoxylin for 1 min and rinsed in water. Slides were then dried and coverslipped with glycerol gelatin.

Tissues from the control fish kidney were assessed for presence of stain for GGT activity and preservation of morphology with respect to post-fixation technique. Differences in staining and morphology between the control and treated fish were also assessed within each technique. Paraffin embedded sections stained with H&E were used to correlate GGT staining differences with tissue pathology.

Expt 3: staining for BrDU uptake. Eighteen adult tilapia, ranging in length from 13.5 to 16.5 cm and in weight from 33 to 82 g, were arbitrarily divided into 2 groups of 9 fish each. The control group received a single IP injection of 10 ml kg⁻¹ sterile water. The treated group received 25 mg kg⁻¹ gentamicin as a single IP injection of 10 ml kg⁻¹ of a 2.5 mg ml⁻¹ solution of gentamicin in sterile water. Food was withheld following injection, and fish were maintained in established 50 gallon (190 l) aquaria with flow-through systems of filtered tap water at a stocking density of 6 or fewer fish per aquarium.

Two fish from each group were sampled at 4, 7, and 14 d following injection and treated with an IP injection of 10 ml kg⁻¹ of a 10 mg ml⁻¹ solution of BrDU (Sigma Chemical Co.). Fish were euthanized 4 h following BrDU injection. Kidneys were removed and fixed in phosphate buffered 10% formalin. Fixed tissue was routinely processed for paraffin sectioning. Sections (6 µm) were cut and 2 slides prepared from each sample. One slide was stained with routine H&E and the other was immunohistochemically stained

for BrDU (de Fazio et al. 1987, Reimschuessel et al. 1990). The BrDU and H&E sections were compared to identify areas of cell replication within collecting duct epithelium, among regenerating epithelial cells along the basement membrane of damaged tubules, and within developing nephrons. Differences in BrDU-uptake between control and treated fish at each time period were assessed for the degree, locality, and chronology of cellular division following injection.

RESULTS

Expt 1: gentamicin exposure

Clinical signs of toxicity (including anorexia, lethargy, exophthalmia, and abdominal distention) developed in the high dose group 2 d after injection, increased in severity until Day 5, and then regressed. No clinical signs were observed in the control or low dose fish. The mortality pattern for all 3 groups following IP injection is shown in Fig. 1. No mortality occurred after Day 5 post-injection. Total mortality during the entire course of this experiment was 0% for the control group, 1.3% for the low dose group, and 29.9% for the high dose group.

The caudal kidney from control fish exemplified normal renal histological structure (Fig. 2A). The tilapia kidney was similar to that of other fish species in that it was not organized into cortical and medullary regions like mammalian kidneys. Glomeruli, proximal and distal convoluted tubules, and collecting ducts, each presenting normal structural features, were found scattered throughout the caudal kidney (Sakai 1985). The normal tilapia kidney differs from that of many other fish species in its relative lack of hemopoietic and immuno-

Fig. 1. *Oreochromis nilotica*. Expt 1: daily mortality of tilapia as a percentage of the population at risk (n = 90) following a single intraperitoneal (IP) injection of a low dose (5 mg kg⁻¹) or high dose (25 mg kg⁻¹) of gentamicin

Fig. 2. *Oreochromis nilotica*. Expt 1: light micrographs of tilapia kidney tissue stained with H&E. (A) 320 $\times$, control. A glomerulus (G) with a basophilic neck segment extending downward to the left of the glomerulus. A small collecting duct is visible directly to the left of the glomerulus. (B) 300 $\times$, 1 d following IP injection of high dose (25 mg kg⁻¹) gentamicin. Numerous necrotic (N) proximal tubules are visible. (C) 300 $\times$, 4 d following injection of high dose gentamicin. The necrotic epithelium has sloughed. Flattened, basophilic epithelial cells recolonize the basement membrane (arrow). (D) 600 $\times$, 14 d following injection of high dose gentamicin. Next to a mature glomerulus (G), a basophilic-staining, developing nephron with a small glomerulus (g) and associated coils of tubule (t) is found connected to a mature collecting duct (c). Note the differences in staining and glomerular size

logic tissue in the renal interstitium of the caudal kidney. Kidney tissue sections were examined for 5 histopathological changes: interstitial edema (increased interstitial space between basement membranes), tubular dilation (increased tubule diameter from that found in normal tissue), necrosis, regeneration along the basement membrane, and nephroneogenesis.

Mild edematous changes (Fig. 2C, D) and moderate tubular dilation (Fig. 2C) were observed in all fish. Statistically significant increases in edema were found only in the Day 2, high dose group (Fig. 3). No significant differences in tubular dilation were found between groups or time periods (Fig. 4). The degree of dilation and edema appeared to be exceedingly variable and relatively mild in all groups.

Grading of renal tubular necrosis was based upon the degree and distribution of active cellular necrosis within the tubules. The most severe cases of tubular necrosis were found in the high dose group and presented cellular pyknosis and detachment of epithelial cells from the basement membrane in every proximal tubule (Fig. 2B). Significant increases in renal tubular necrosis were found in the high dose group from Days 1 to 4, and in the low dose group at Days 4 and 7 (Fig. 5).

Regeneration along the tubular basement membrane was a characteristic sequel to profound tubular necrosis. Regenerating tubules were observed as basement membrane structures which partially or completely lacked normal tubular epithelium and were populated instead by small, flattened, basophilic (actively replicating) cells (Fig. 2C). Significant increases in the degree of regeneration along the tubular basement membrane were evident only in the high dose group at 3, 4, 14 and 21 d after gentamicin exposure (Fig. 6).

Nephroneogenesis was characterized by the appearance of small, intensely basophilic clusters of

Fig. 3. *Oreochromis nilotica*. Expt 1. mean severity of renal interstitial edema following a single IP injection of a low dose (5 mg kg^{-1}) or high dose (25 mg kg^{-1}) of gentamicin. a: statistically greater than control and low dose groups ($p < 0.05$)

Fig. 4. *Oreochromis nilotica*. Expt 1. mean severity of renal tubular dilation following a single IP injection of a low dose (5 mg kg^{-1}) or high dose (25 mg kg^{-1}) of gentamicin

cells developing into small tubules and rudimentary glomeruli. Occasionally these new nephrons could be found adjacent to, and apparently invading, collecting ducts (Fig. 2D). The number of developing nephrons per unit area of tissue section was used to quantify nephroneogenesis, and a significant increase in nephroneogenesis was evident only in the high dose group at Day 14 following injection (Fig. 7). Both high and low dose groups displayed decreases in number of new nephrons mm^{-2} at varying time periods. The low dose group had a significant decrease in nephroneogenesis at 1 and 2 d following gentamicin exposure. The high dose group had a significant decrease in nephroneogenesis from 1 to 4 d following injection (Fig. 7). No significant decreases or increases were found in the control group at any time.

Fig. 5. *Oreochromis nilotica*. Expt 1. mean severity of renal tubular necrosis following a single IP injection of a low dose (5 mg kg^{-1}) or high dose (25 mg kg^{-1}) of gentamicin. a: statistically greater than control group only ($p < 0.05$); b: statistically greater than control and low dose groups ($p < 0.05$); c: statistically greater than control and high dose groups ($p < 0.05$)

Fig. 6. *Oreochromis nilotica*. Expt 1: mean degree of renal tubular regeneration following a single IP injection of a low dose (5 mg kg^{-1}) or high dose (25 mg kg^{-1}) of gentamicin. a: statistically greater than control group only ($p < 0.05$); b: statistically greater than control and low dose groups ($p < 0.05$)

Expt 2: staining for GGT activity

Staining for GGT enzyme was successful in all control frozen slide samples, regardless of the post-fixative used. Intensity of staining was greatest in the ethanol post-fixed slides and most attenuated in the formalin post-fixed slides. The preservation of tissue morphology was poorest in those slides which received no post-fixation, marginal in the slides post-fixed with acetone, and reasonably good in slides post-fixed with ethanol or formalin. The best combination of staining and morphology preservation was obtained with post-fixation of the frozen tissue slides with chilled 95% ethanol for 1 min.

Examination for GGT staining of ethanol post-fixed slides from the control fish revealed intense staining of the enzyme marker around the renal tubular lumen and in the epithelial cell brush border (Fig. 8A). Slides from the treated fish showed no staining for GGT activity in the tubules, and disruption of the tubular morphology (Fig. 8B). Tissue samples stained with H&E revealed severe necrosis and sloughing of renal tubular epithelium in the treated fish in comparison to the normal renal appearance of the control fish.

Expt 3: staining for BrDU uptake

Immunohistochemical staining of the treated fish tissues for BrDU uptake revealed an increase in cellular replication in various parts of the kidney over normal control levels (Fig. 9A). At 4 d following injection, replication was evident primarily in the large terminal collecting ducts (Fig. 9B). At 7 d, some activity in the collecting ducts was still evident but activity was also prominent in flattened epithelial cells on the pre-

Fig. 7. *Oreochromis nilotica*. Expt 1: number of developing nephrons mm^{-2} of renal tissue section following a single IP injection of a low dose (5 mg kg^{-1}) or high dose (25 mg kg^{-1}) of gentamicin. a: statistically greater than control group only ($p < 0.05$); b: statistically less than the high dose group as a whole ($p < 0.001$); c: statistically less than the low dose group as a whole ($p < 0.05$)

dominantly denuded tubule basement membranes (Fig. 9C). By 14 d following injection, cellular division in the collecting ducts was greatly decreased, but division among tubular epithelial cells was increased. Division was also evident in clusters of cells identified on H&E stained sections as developing nephrons (Fig. 9D). Some clusters of basophilic staining cells were noted in both control and treated tissues which did not stain for BrDU. These were presumed to be inactive precursors to new nephrons.

DISCUSSION

Consistent with evidence found in other fish species (Chamie & Reimschuessel 1994, Reimschuessel & Williams 1994, Rokokous et al. 1994), tilapia were shown to be susceptible to nephrotoxic injury from exposure to gentamicin. A single injection of 5 mg kg^{-1} , less than double the recommended therapeutic dose of 3 mg kg^{-1} (Stoskopf et al. 1986), was sufficient to induce marked necrosis and a low level of mortality in this species. A single injection of 25 mg kg^{-1} (approximately 8 times the recommended therapeutic dose) produced severe necrosis of the renal tubular epithelium and significant mortality. Tilapia are thus lethally more sensitive to the nephrotoxic effects of gentamicin than are the goldfish, in which a dose of 50 mg kg^{-1} did not produce significant mortality (Reimschuessel & Williams 1994, Rokokous et al. 1994).

Mild and highly variable levels of interstitial edema and tubular dilation, 2 signs commonly associated with renal injury, were evident across all groups in this investigation. Only the high dose group, on Day 2,

Fig. 8. *Oreochromis nilotica*. Expt 2: light micrographs of tilapia tissue stained for gamma glutamyl transpeptidase activity. (A) 300 $\times$, control, 3 d following IP injection with sterile water. Note the dense staining around the tubular lumen coinciding with the brush border of the proximal tubular epithelium. (B) 300 $\times$, 3 d following injection of 25 mg kg⁻¹ gentamicin. Note the loss of staining and the degree of morphological disruption compared with the control

showed sufficiently severe edema to be determined significant from the background levels of edematous change. This finding might be partially explained by the presence of mild hypoxic renal injury as a result of the method of euthanasia. The anesthetic agent MS-222, utilized for euthanasia in this experiment, has been demonstrated to cause acute, severe hypoxia at narcotic doses (Iwama et al. 1989). This hypoxia may have resulted in mild, variable, background levels of interstitial edema and tubular dilation in all of the groups of this experiment.

The high dose group had the highest incidence and severity of necrosis with the peak necrotic changes occurring at Day 2 following injection. Peak necrosis was delayed in the low dose group until Days 4 to 7, and never reached the degree noted in the high dose group. The dramatic decrease in necrosis after peak levels were reached (Fig. 5) resulted from the complete obliteration of the renal tubular epithelium, which left denuded tubular basement membranes and no further cells to undergo active cellular necrosis. Regeneration along the basement membrane closely followed peak necrosis. The denuded basement membranes were rapidly, if sparsely, populated by flattened, basophilic epithelial cells. The low dose group had a low degree

of epithelial regeneration that was consistent with the milder degree of tubular necrosis. The high dose group showed a high degree of regeneration that peaked at Day 4, closely following the peak in necrosis seen at Day 2, and continued through Day 21 following exposure. Regeneration thus appeared to begin shortly after peak necrosis and continue for at least 2 wk before returning to background levels.

Nephroneogenesis developed more slowly than necrotic or regenerative processes, first exceeding background levels and simultaneously peaking at Day 14 following injection. A greater number of new nephrons developed in the high dose group than in the low dose group, again consistent with the relative degree of necrosis. The developing nephrons seen at 14 d were relatively mature, showing rudimentary glomeruli and tubules. There also appeared to be a trend for decreased nephroneogenesis during the period in which active necrosis and repair took place. A moderate level of nephroneogenic activity was present in all groups at 0.25 and 0.5 d, followed by a decrease in activity in both high and low dose groups (in some cases a significant decrease from the overall group level) between Days 1 and 4, and then a return to moderate activity following the peak nephroneo-

Fig. 9. *Oreochromis nilotica*. Expt 3: light micrographs of tilapia kidney tissue stained for uptake of 5-bromo-2'-deoxyuridine (BrDU). (A) 300 $\times$, control, 4 d following IP injection of sterile water. Note the single nucleus in the large collecting duct that demonstrates BrDU uptake (arrow). (B) 300 $\times$, 7 d following injection of 25 mg kg⁻¹ gentamicin. The large collecting duct contains numerous nuclei with staining for BrDU uptake. A large amount of cellular debris from necrosis of proximal tubular epithelium is visible in the lumen of the collecting duct. (C) 300 $\times$, 14 d following injection of 25 mg kg⁻¹ gentamicin. BrDU staining is found in numerous flattened cells (arrows) colonizing the otherwise denuded tubule basement membrane. (D) 600 $\times$, 14 d following injection of 25 mg kg⁻¹ gentamicin. Intense BrDU staining, indicating pronounced proliferation, is found in a basophilic cluster of cells of an immature developing nephron (DN)

genesis at 14 d (Fig. 7). This trend may indicate that nephrotoxic injury suppresses the normal background level of nephroregeneration in this species, and then stimulates a responsive burst of new nephron development once active necrosis has ceased and regeneration along the tubules is under way.

GGT is commonly found in the brush border of renal proximal tubules (Rutenburg et al. 1969, Reimschuessel et al. 1989). Loss of GGT staining was positively correlated with the histopathological appearance of tubular necrosis in gentamicin-treated fish, lending strong evidence that the proximal tubule is the target of gentamicin nephrotoxicity in this species. The sensitivity of tilapia GGT to inactivation by formalin has not been reported in GGT from goldfish (Reimschuessel et al. 1989) and may indicate structural differences in this enzyme in goldfish and tilapia.

This investigation demonstrated that the tilapia kidney responds to nephrotoxic insult with regeneration of epithelial cells along the tubule and new nephron development in a manner similar to other fish species (Cuppage & Tate 1967, Reimschuessel et al. 1990, 1993, Reimschuessel & Williams 1994, Rokokous et al. 1994). The time course for regeneration along the basement membrane appeared to be similar in Expts 1 & 3 of this study with regeneration commencing in both by Day 4 and continuing beyond Day 14. Expt 3 utilized BrDU uptake as a marker for cellular replication and demonstrated the chronology of the process of epithelial regeneration. Cellular division began in the collecting ducts shortly after nephrotoxic insult, progressed to regenerative cells migrating along the denuded basement membrane, and later tapered off in the collecting ducts while continuing in the cells lining the damaged tubules. This chronology may indicate that the collecting ducts are the source of replicating cells for repopulation of the proximal tubular epithelium.

A marked difference was noted in the quality of nephroregeneration in fish from Expt 3 compared to that in Expt 1. The developing nephrons found at Day 14 in Expt 3 were more immature, appearing as little more than clusters of rapidly dividing cells (Fig. 9D), while those in Expt 1 were more complex (Fig. 2D). This apparent disparity in the quality of new nephron development may be explained by the fact that the fish utilized in Expt 1 were less mature than those in Expt 3. A certain background level of new nephron development is probably normal in fish that have not reached maturity. Immature fish may respond to nephrotoxic insult with new nephron development more rapidly than adults because of this background level of ongoing nephroregeneration. Future investigation on the effect of fish age or size upon the rate of new nephron development is indicated. Investigations

using electron microscopy to document the ultrastructural changes involved in nephron regeneration and neogenesis would also be interesting.

This investigation demonstrated that *Oreochromis nilotica* is more sensitive to the nephrotoxic effects of gentamicin than other previously examined non-tilapia species in that the tilapia experienced nearly 30 % mortality at 25 mg kg⁻¹ gentamicin whereas goldfish experience no mortality at the same dose (Reimschuessel & Williams 1994, Rokokous et al. 1994). This has important implications for therapeutic use of gentamicin in tilapia fishes. Dosing must be approached cautiously if toxicity and mortality are to be avoided. Although tilapia are able to regenerate injured tubular epithelium and generate new nephrons in response to renal injury, acute renal damage may result in mortality before the regenerative responses can be initiated. A tilapia fish that is otherwise compromised by stresses such as bacterial infection, crowding, or poor water quality may be less well equipped to repair tubular injury resulting from the nephrotoxic insult of aminoglycoside therapy and thus be more likely to succumb. Such stresses are particularly prevalent among tilapia in commercial aquaculture. Of further note, all nephrotoxic injury induced in this investigation resulted from a single dose exposure, while most clinical therapy involves repetitive dosing regimes. Clearly, further investigation on the effects of repetitive dosing of gentamicin on the severity of nephrotoxicity in tilapia is warranted, as it would be helpful in determining the suitability of the clinical use of gentamicin antibacterial therapy in commercial tilapia culture.

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