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Compensatory Renal Hypertrophy and the Handling of an Acute Nephrotoxicant in a Model of Aging

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Abstract

Aging often results in progressive losses of functioning nephrons, which can lead to a significant reduction in overall renal function. Because of age-related pathological changes, the remaining functional nephrons within aged kidneys may be unable to fully counteract physiological and/or toxicological challenges. We hypothesized that when the total functional renal mass of aged rats is reduced by 50%, the nephrons within the remnant kidney do not fully undergo the functional and physiological changes that are necessary to maintain normal fluid and solute homeostasis. We also tested the hypothesis that the disposition and handling of a nephrotoxicant is altered significantly in aged kidneys following an acute, 50% reduction in functional renal mass. To test these hypotheses, we examined molecular indices of renal cellular hypertrophy and the disposition of inorganic mercury (Hg^{2+}), a model nephrotoxicant, in young control, young uninephrectomized (NPX), aged control and aged NPX Wistar rats. We found that the process of aging reduces the ability of the remnant kidney to undergo compensatory renal growth. In addition, we found that an additional reduction in renal mass in aged animals alters the disposition of Hg^{2+} and potentially alters the risk of renal intoxication by this nephrotoxicant. To our knowledge, this study represents the first report of the handling of a nephrotoxicant in an aged animal following a 50% reduction in functional renal mass.

1.0. Introduction

Normal aging often leads to substantial pathological changes in the kidneys, which can significantly reduce the number of functioning nephrons. Glomeruli are often affected first and are characterized by thickened basement membranes, expanded mesangial matrices, shrinkage and occlusion of the glomerular capillaries, and eventual complete glomerulosclerosis (Choudhury 2004; Lopez-Novoa 2008; Zhou 2008; Zhou et al. 2008). Additional pathological changes in the kidneys may also occur as the result of one or more disease states. Diabetes and hypertension are common in individuals over the age of 65 and may cause additional reductions in the number of functioning nephrons (CDC 2013; Davis

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et al. 2011; NIDDK 2012), which may eventually lead to varying degrees of renal insufficiency.

In aged individuals (> 80 years), the renal functional reserve has been shown to be reduced significantly (Esposito et al. 2007). When the functional renal mass in elderly (> 65 years) and aged (> 80 years) individuals is reduced further by pathological or toxicological challenges, it is likely that the remaining functional renal mass is incapable of maintaining normal fluid and solute homeostasis. It is important to note that when young healthy kidneys are challenged by mild to modest reductions in functional renal mass, compensatory changes including cellular hypertrophy, increased cellular metabolism, and hyperfiltration occur in the kidneys in an attempt to maintain fluid and solute balance in extracellular compartments (Fine and Norman 1989). Given the reduction in functional renal reserve, caused by both aging and disease, it is possible that aged kidneys are unable to undergo the compensatory morphological and functional changes that would occur normally under these circumstances in the kidneys of younger individuals.

An important health concern related to aged individuals is environmental and/or occupational exposure to nephrotoxics. Aged individuals may be exposed to various nephrotoxics over the course of their lifetimes, which could lead to significant accumulation of injurious elements/chemicals in tissues and organs (Bridges 2013). Aged individuals with diminished renal function caused by age and/or disease may experience alterations in the handling of nephrotoxics and thus, these individuals may be at a greater risk of intoxication than younger individuals who possess a greater capacity to eliminate these toxicants. Since the early signs of renal insufficiency and chronic kidney disease (CKD) often go undetected and many individuals are not diagnosed until symptoms of uremia are manifested, individuals may continue to be exposed to nephrotoxics in the early stages of CKD. This continued exposure may enhance morbidity and even mortality. Owing to the growing population of aged individuals, the incidence of diabetes and hypertension in this population, and the prevalence of nephrotoxics in occupational and environmental settings, it is important that we have a thorough understanding of how aged kidneys with and without additional significant reductions in functioning nephrons handle nephrotoxics.

In the current study, we used inorganic mercury (Hg^{2+}) as a model nephrotoxicant since the renal effects and disposition of Hg^{2+} have been studied extensively and are well-characterized (Bridges and Zalups 2010; Clarkson 1993; Clarkson and Magos 2006; Zalups 2000). We utilized uninephrectomized (NPX) rats as a model of reduced renal mass, which in young adults, does not compromise fluid and electrolyte homeostasis (Rodriguez-Gomez et al. 2012). We designed this study to 1) test the hypothesis that the remnant kidney of aged rats lacks the ability to fully undergo the compensatory hypertrophic changes that occur normally in young and middle-aged adults after an acute, 50% reduction of renal mass; and 2) test the hypothesis that the disposition and handling of a non-toxic dose of Hg^{2+} are altered in the aged remnant kidney after an acute 50% reduction of renal mass.

2.0. Methods

2.1. Animals

Male Wistar rats were obtained from our breeding colony housed in the Mercer University School of Medicine animal facility. “Young” adult rats were used at an age of eight weeks while “Aged” rats were approximately 20 months of age. Mean body weights for each group of animals are listed in Table 1. Animals were provided a commercial laboratory diet (Teklad Global Soy Protein Free Extruded Rodent Diet, Harlan Laboratories) and water *ad libitum* throughout all aspects of the present study. All experimental protocols involving animals were approved by the Mercer University Institutional Animal Care and Use Committee. Animals were handled in accordance with the Guide for the Care and Use of Laboratory Animals as adopted by the National Institutes of Health.

2.2. Surgery

Rats were anesthetized with an intraperitoneal (i.p.) injection of 70 mg kg⁻¹ ketamine and 6 mg kg⁻¹ xylazine following which, an incision was made through the skin and musculature in the right flank. Subsequently, the kidney was isolated from the perinephric fascia and the renal artery, renal vein, and the ureter were ligated with 4-0 silk suture. The right kidney was removed without damaging the liver or corresponding adrenal gland. Control animals were not subjected to surgical procedures since previous studies indicate that there is no significant difference between surgical and non-surgical controls (Lash et al. 1999).

2.3. Intravenous Injections

Rats were injected intravenously (i.v.) with a non-nephrotoxic (0.5 μmol kg⁻¹ 2 mL⁻¹ normal saline) dose of HgCl₂ according to our previously published protocol (Bridges et al. 2008a; b). The injection solution contained radioactive mercury ([²⁰³Hg²⁺]) and was designed to deliver 1 μCi [²⁰³Hg²⁺] to each animal. [²⁰³Hg²⁺] was generated by neutron activation of mercuric oxide for four weeks at the University of Missouri Research Reactor (MURR) (Belanger et al. 2001; Bridges et al. 2008a). At the time of injection, each animal was anesthetized with isoflurane and a small incision was made in the skin in the mid-ventral region of the thigh to expose the femoral vein and artery, following which the dose of HgCl₂ was administered into the vein. Injection into the femoral vein is preferred over the tail vein because there is less back-leak of Hg. The wound was closed using two 9-mm stainless steel wound clips. Animals were then housed individually in metabolic cages. Twenty-four hours after injection with HgCl₂, animals were euthanized and organs/tissues were harvested.

2.4. Collection of Organs and Tissues

At the time of sacrifice, animals were anesthetized with an i.p. injection of ketamine (70 mg kg⁻¹) and xylazine (30 mg kg⁻¹). A 1-mL sample of whole blood was obtained from the inferior vena cava and set aside for determination of Hg²⁺ content. A separate sample of blood was placed in a Microtainer plasma separation tube in order to estimate content of Hg²⁺ in the plasma and cellular fractions. The total volume of blood was estimated to be 6% of body weight (Lee and Blafox 1985).

The kidneys were also removed from each rat. Each kidney was trimmed of fat and fascia, weighed, and cut in half along the mid-transverse plane. In control animals, one-half of the right kidney was placed in fixative (40% formaldehyde, 50% glutaraldehyde in 96.7 mM NaH_2PO_4 and 67.5 mM NaOH) for future histological analyses. The remaining half was frozen in liquid nitrogen for future RNA and oxidative stress analyses. One-half of the left kidney was utilized for estimation of Hg^{2+} content. A 3-mm transverse slice was obtained from the remaining half and was used for dissection of renal zones (cortex, outer stripe of the outer medulla (OSOM), inner stripe of the outer medulla (ISOM), and inner medulla). Since NPX animals lacked a right kidney that could be used for histological and RNA analyses, each group of NPX rats (young and aged) was separated randomly into two groups of four rats per group. In the first group, one-half of the left kidney was utilized for estimation of Hg^{2+} content. A 3-mm transverse slice was obtained from the remaining half and was used for the dissection of renal zones. The remaining piece of kidney was utilized for histological analyses. In the second group of NPX rats, one-half of the left kidney was utilized for estimation of Hg^{2+} content while the remaining half was used for dissection of renal zones and RNA and oxidative stress analyses. Each sample was weighed and placed in a separate tube for estimation of Hg^{2+} . The liver was excised, weighed, and a 1-g sample was set aside for determination of Hg^{2+} content.

Urine and feces were collected throughout the 24-hour the experiment. The total volume of urine excreted by each animal was weighed and the volume was recorded. A 1-mL sample was then weighed and placed in a tube for estimation of Hg^{2+} content. All of the feces excreted during the 24-hour experiment were counted for estimation of Hg^{2+} content.

The content of [$^{203}\text{Hg}^{2+}$] in each sample was determined by counting in a Wallac Wizard 3 automatic gamma counter (Perkin Elmer, Boston, MA). Standard computational methods were used to determine the content of Hg^{2+} in each sample.

2.5. Real-time PCR

At the time of RNA isolation, frozen samples of kidney were pulverized with a mortar and pestle. TRIzol Reagent (Life Technologies, Grand Island, NY) was added to each ground sample and RNA was extracted according to the manufacturer's protocol.

Reverse transcription of 1 μg of RNA was carried out using reverse transcriptase and random hexamers (Life Technologies). For real-time PCR analyses, 2 μL of the reverse transcriptase reaction were utilized. Analyses of Na^+ , K^+ -ATPase (*Atp1a4*; Rn00575682_m1), organic anion transporter 1 (*Scl22A6*; Rn00568143_m1), $\text{b}^{0,+}\text{AT}$ (*Scl7a9*; Rn00588400_m1), and vascular endothelial growth factor (*Vegfa*; Rn00582935_m1), glutamate cysteine ligase (*gclc*; Rn00689046_m1) were performed with an ABI Prism 7000 detection system using a Gene Expression Assays (Life Technologies). Glyceraldehyde 3-phosphate dehydrogenase (*Gapdh*; Rn01775763_g1) was used as a reference gene. Samples from each kidney were processed in triplicate.

2.6. Morphometric Analyses

Renal tissue from each rat was fixed in 40% formaldehyde, 50% glutaraldehyde in 96.7 mM NaH_2PO_4 and 67.5 mM NaOH for 48 hours at 4°C. Following fixation, kidneys were washed twice with normal saline and placed in 70% ethanol. Tissues were processed in a Tissue-Tek VIP processor using the following sequence: 95% ethanol for 30 min (twice); 100% ethanol for 30 min (twice); 100% xylene (twice). Tissue was subsequently embedded in POLY/Fin paraffin (Fisher) and 5 μm sections were cut using a Leitz 1512 microtome and mounted on glass slides. Sections were stained with hematoxylin and eosin (H & E) and were viewed using an Olympus IX70 microscope. The diameters of proximal tubules were measured using an eyepiece reticle calibrated with a stage micrometer. A section of kidney was analyzed from four rats within each group. For each section, the diameters of proximal tubules (approximately 8-10) were measured within four randomly selected 400x microscopic fields. The mean tubular diameter was calculated from the four fields on each slide and a mean of means ($n = 4$) was calculated for each group of rats.

2.7. Measurement of Plasma Creatinine

Plasma creatinine levels were assessed in order to estimate alterations in glomerular filtration rate (GFR). Following separation of plasma from cellular components of blood, plasma samples were stored at -20°C. For determination of plasma creatinine, 30 μL of plasma were utilized and the concentration of creatinine was assessed using the QuantiChrome creatinine assay (BioAssay). Each sample was processed in triplicate according to the manufacturer's protocol and plates were read in a Tecan spectrophotometer at 510 nm.

2.8. Assessment of Oxidative Stress

Lipid peroxidation is widely regarded as a biomarker of oxidative stress (Niki 2008). 4-hydroxynonenal (4-HNE) is a by-product of lipid peroxidation and is an accepted measure of oxidative stress (Shoeb et al. 2014). In the current study, 4-HNE was measured in frozen samples of renal tissue from rats using the OxiSelect HNE Adduct Competitive ELISA kit from Cell Biolabs, Inc. Samples were processed according to the manufacturer's protocol and were read in a Tecan spectrophotometer at 450 nm.

2.9. Statistical Analyses

Corresponding sets of data for each parameter assessed were analyzed first with the Kolmogorov-Smirnov test for normality and then with Levene's test for homogeneity of variances. All data passed the preliminary tests and were subsequently analyzed using a two-way analysis of variance (ANOVA) to assess differences among the means. When statistically significant F -values were obtained with ANOVA, differences among means were analyzed using Tukey's *post hoc* multiple comparison test. A P -value of 0.05 was considered statistically significant. Groups of control animals contained five rats per group while groups of NPX animals contained seven rats per group. Four randomly selected microscopic fields (400x) in each histological section were examined for the morphometric studies.

3.0. Results

3.1. Histological and Morphological Analyses

The mean weight of the left kidneys from each group of animals is shown in Table 1. The combined total renal mass at the time of euthanasia is also shown in Table 1. The gross appearance of kidneys from young control and young NPX rats was normal. In contrast, kidneys from aged control and aged NPX rats appeared discolored and mottled. Histologically, kidneys from young control animals were normal. In young NPX rats, the lumens of proximal tubules appeared dilated and tubular epithelial cells appeared enlarged. No other histological alterations were obvious. In aged control rats, pathological alterations such as glomerulosclerosis, interstitial fibrosis, and proteinaceous deposits were observed as reported previously (Bridges et al. 2014). The kidneys of aged NPX rats were similar histologically to that of aged control rats (data not shown).

The outer diameters of proximal tubules in the cortex and OSOM from each group of rats were measured (Figure 1). The outer diameter of proximal tubules in the remnant kidney of young NPX rats was significantly greater than that of proximal tubules in the other three groups of rats. There was no significant difference in the outer diameter of proximal tubules among groups of young control, aged control, and aged NPX rats.

3.2. RT-PCR Analyses

The renal expression of Na^+ , K^+ -ATPase, Oat1, $\text{b}^{0,+}\text{AT}$, and Vegf was measured in each group of rats as a means to assess the manifestation of compensatory renal hypertrophy at the molecular level. The renal expression of Na^+ , K^+ -ATPase (Figure 2A) was significantly greater in young NPX rats than in young control rats. In aged rats, the expression of Na^+ , K^+ -ATPase in NPX rats and control rats was not significantly different. There was no significant difference in the renal expression of Na^+ , K^+ -ATPase between young control and aged control rats.

The renal expression of Oat1 was significantly greater in young NPX rats than in young control rats (Figure 2B). In aged rats, there was no significant difference in the renal expression of Oat1 between control and NPX rats. Interestingly, the expression of Oat1 in the kidneys of aged rats (control and NPX) was significantly lower than that in kidneys of corresponding young rats.

The expression of $\text{b}^{0,+}\text{AT}$ was measured in kidneys from each group of rats (Figure 2C). The expression of $\text{b}^{0,+}\text{AT}$ was significantly greater in the remnant kidney of young NPX rats than that in the normal kidneys of young control rats. The expression of $\text{b}^{0,+}\text{AT}$ in aged control and aged NPX rats was not significantly different from that in young NPX rats.

The expression of Vegf in the remnant kidney of young NPX rats was significantly greater than that in normal kidneys of young control rats (Figure 2D). There was no significant difference in the renal expression of Vegf between aged control and aged NPX rats. Furthermore, the renal expression of Vegf in aged control and aged NPX rats was significantly lower than that of young control rats.

3.2. Measurements of oxidative stress

The formation of 4-HNE adducts is a reliable measurement of oxidative stress (Dubinina and Dadali 2010). The measured concentration of adducts ($\mu\text{g/mL}$) in the kidneys of young control rats was not significantly different from that in the remnant kidney of young NPX rats (Figure 3A). In contrast, the formation of 4-HNE adducts in the kidneys of aged control rats was significantly greater than that in the kidneys of young rats (control or NPX). Furthermore, the adduct formation in the remnant kidney of aged NPX rats was significantly greater than that in the renal tissue of all other groups.

Real-time PCR analyses were used to assess the expression of mRNA encoding Gcl, the rate-limiting enzyme in glutathione (GSH) biosynthesis. The expression of Gcl in the remnant kidney of young NPX rats was significantly greater than that in normal kidneys of young control rats (Figure 3B). There was no significant difference in the renal expression of Gcl between aged control and aged NPX rats. However, the renal expression of Gcl in aged control and aged NPX rats was significantly lower than that of corresponding groups of young rats.

3.3. Plasma Creatinine

Plasma creatinine (Figure 4) was measured as a means of evaluating the average glomerular filtration rate in each group of rats. The concentration of plasma creatinine in the group of young control rats was similar to that reported previously for normal rats (Amini et al. 2012; Moeini et al. 2013). The concentration of plasma creatinine in young NPX rats was not significantly different from that in young control rats. However, as reported previously (Bridges et al. 2014), the concentration of plasma creatinine in aged control rats was significantly greater than that of young control rats. In addition, the concentration of plasma creatinine in aged NPX rats was significantly greater (about three-fold) than that of young control and young NPX rats.

3.4. Disposition of Hg^{2+}

Figure 5 shows the renal concentration of Hg^{2+} (% administered dose g^{-1}) in young and aged rats exposed to $0.5 \mu\text{mol HgCl}_2 \text{ kg}^{-1}$. The renal concentration of Hg^{2+} was significantly greater in young rats than in aged rats. In both young and aged rats, the renal concentration of Hg^{2+} was significantly greater in the remnant kidney of NPX animals than in the kidneys of corresponding control animals.

Figure 6A shows the concentration of Hg^{2+} (% administered dose g^{-1}) in the renal cortex of young and aged rats exposed to $0.5 \mu\text{mol HgCl}_2 \text{ kg}^{-1}$. The concentration of Hg^{2+} in the renal cortex was significantly greater in the renal cortex of young rats than in that of aged rats. In young rats, there was no significant difference in the cortical concentration of Hg^{2+} between NPX and control animals. In aged rats, the renal cortical concentration of Hg^{2+} was significantly greater in the NPX rats than in control rats. Figure 6B shows the concentration of Hg^{2+} in the renal outer stripe of the outer medulla (OSOM). There were no significant differences in the concentration of Hg^{2+} in the OSOM between corresponding groups of young and aged rats (either control or NPX). However, the concentration of Hg^{2+} in the

OSOM in both young and aged rats was significantly greater in the NPX rats than in the control rats.

The amount of Hg^{2+} excreted in urine during the initial 24 h after exposure is shown in Figure 7. The urinary excretion of Hg^{2+} was significantly greater in aged rats (control or NPX) than in young rats. There were no differences in the urinary excretion of Hg^{2+} between control and NPX rats in the young or aged groups.

The hepatic burden of Hg^{2+} (% administered dose) is shown in Figure 8A. The amount of Hg^{2+} in the liver of young control and NPX rats was not significantly different from that of corresponding groups of aged rats. However, the amount of Hg^{2+} detected in the liver of young NPX rats was significantly greater than that in the liver of young control rats. The hepatic burden of Hg^{2+} in aged control rats was not significantly different from that of aged NPX rats.

The fecal excretion of Hg^{2+} is shown in Figure 8B. The amount of Hg^{2+} excreted in the feces by young NPX rats during the 24-hour experiment was significantly greater than that excreted by young control rats. In contrast, the amount of Hg^{2+} excreted in feces by aged NPX rats was significantly lower than that excreted by aged control rats. In general, the amount of Hg^{2+} excreted in the feces by aged rats was significantly lower than that excreted by corresponding young rats.

The amount of Hg^{2+} in the total volume of whole blood of young and aged rats exposed to $0.5 \mu\text{mol HgCl}_2 \text{ kg}^{-1}$ is shown in Figure 9. The burden of Hg^{2+} in blood of aged NPX rats was significantly greater than that of young NPX rats. There was no difference in the hematologic burden of Hg^{2+} among the other groups of rats.

4.0. Discussion

The current study was designed to test the hypothesis that aged kidneys have a reduced ability to undergo the compensatory changes that are necessary to maintain normal fluid and solute homeostasis following an additional reduction of functional renal mass. In addition, since aged individuals may be exposed to numerous toxicants over their lifetimes, we also tested the hypothesis that the handling and disposition of certain nephrotoxics, such as Hg^{2+} , are altered in aged kidneys following an acute 50% reduction of renal mass.

We first assessed the ability of the remnant kidney of an NPX animal to undergo cellular hypertrophy by comparing the outer diameter of proximal tubules in control and NPX rats. Tubular diameter was significantly greater in young NPX rats than in young control rats suggesting that the proximal tubular cells in the remnant kidney of NPX rats were hypertrophied. This observation corresponds with previously published findings indicating that cellular hypertrophy of proximal tubular cells is a characteristic compensatory change that occurs after a significant loss of functional renal mass (Fine and Norman 1989; Fine 1992). In contrast, the outer diameter of proximal tubules in aged NPX rats was not significantly different from that in aged control rats, suggesting that the cells in the remnant kidney of aged animals have a reduced capacity to undergo cellular hypertrophy following uninephrectomy.

We also examined the presence of compensatory changes at the molecular level by measuring the renal expression of Na⁺, K⁺-ATPase, Oat1, b^{0,+}AT, and Vegf. Since the surface area of the plasma membrane of proximal tubular cells increases significantly following uninephrectomy (Fine 1992), we tested the hypothesis that the expression of membrane-bound transporters also increases. Our real-time PCR data indicate that the renal expression of Na⁺, K⁺-ATPase increases significantly in young rats after uninephrectomy, consistent with the reported features of cellular hypertrophy in proximal tubules (Epstein et al. 1978; Fine and Norman 1989; Fine 1992). In aged rats, the renal expression of Na⁺, K⁺-ATPase was similar between control and NPX rats, suggesting that renal compensatory changes such as increased expression of proteins do not occur in aged animals. This lack of compensatory change may lead to significant alterations in fluid and solute homeostasis, which may lead to alterations in GFR. Interestingly, the expression of Na⁺, K⁺-ATPase was lower in remnant kidneys of aged NPX rats than in that of young NPX rats. This reduced expression is most likely due to tubular atrophy and cellular necrosis that occur in kidneys of aged animals. In addition, the pattern of Oat1 expression was similar to that of Na⁺, K⁺-ATPase. Furthermore, the expression of b^{0,+}AT, which is the light chain subunit of the amino acid transporter, System b^{0,+} localized in the luminal membrane of proximal tubular cells (Pfeiffer et al. 1999), was also enhanced following uninephrectomy in young rats. Taken together, these findings suggest that cellular hypertrophy occurs in the remnant kidney of young NPX rats. Since the expression of these proteins in aged animals did not increase after uninephrectomy, we suggest that proximal tubular segments in aged kidneys are unable to undergo compensatory hypertrophic changes in response to uninephrectomy. Interestingly, the expression of b^{0,+}AT was elevated in kidneys of aged control and aged NPX rats. One possible explanation for this increase is that the expression of luminal amino acid carriers in proximal tubular cells of diseased nephrons with reduced GFR may be enhanced in order to compensate for reductions in filtration fraction and tubular uptake of nutrients. Additional studies are clearly necessary to fully characterize this finding.

In addition to measuring the expression of select membrane transporters in proximal tubular cells, we also examined the renal expression of Vegf, which is localized in the glomeruli and along tubular segments of nephrons (Cooper et al. 1999). Enhanced expression of Vegf has been shown to correlate with compensatory renal hypertrophy following significant reductions in renal mass (Flyvbjerg et al. 2002; Pillebout et al. 2001). Our analyses indicate that the renal expression of Vegf is significantly greater in young NPX rats than in young control rats, indicating that renal cellular hypertrophy occurs in young NPX animals. Importantly, the expression of Vegf in the remnant kidney of aged NPX animals was not different from that in kidneys of aged control animals, suggesting that compensatory cellular hypertrophy does not occur in the remnant kidneys of aged NPX animals.

Owing to the apparent inability of aged kidneys to respond appropriately to additional, acute significant reductions in functional renal mass, we propose that aged individuals may experience added adverse renal effects. Indeed, plasma creatinine in aged rats increased approximately threefold after uninephrectomy. In young rats, however, uninephrectomy did not significantly alter plasma creatinine levels. These findings suggest that aged rats have a reduced ability to undergo the compensatory changes that are necessary to maintain GFR and fluid/solute balance within a normal range. This lack of compensatory change may

relate to reductions in certain growth factors, such as Vegf, that play important roles in the induction of cellular hypertrophy.

Our findings also indicate that aged kidneys may be less able to deal with challenges such as those associated with oxidative stress. In the current study, we measured the presence of HNE adducts as an indicator of oxidative stress. Renal HNE concentrations in aged control rats were significantly greater than those in young control rats, reaffirming previous findings that aging in itself leads to an increase in the baseline level of oxidative stress in the kidneys (Wang et al. 2014). We also found that renal levels of HNE in aged NPX rats were significantly greater than those in aged control rats, suggesting that remnant renal tissue of aged animals lacks the ability to effectively counteract oxidative stress that occurs following a significant loss of functional renal mass. Moreover, we found that the renal expression of Gcl, which is the rate-limiting enzyme in GSH synthesis, was significantly reduced in aged NPX animals. This reduction may contribute to the increased levels of oxidative stress detected in these animals. Gcl expression was also enhanced in young NPX animals, which has been shown previously to be a characteristic of renal cellular hypertrophy (Lash et al. 2001; Lash and Zalups 1994).

In order to assess the effects of aging and uninephrectomy on the handling of a nephrotoxicant, we examined the disposition of Hg^{2+} in various organs of aged and young (control and NPX) rats. The renal concentration of Hg^{2+} (% administered dose g^{-1}) was significantly greater in young rats than in aged rats. The reduced renal accumulation of Hg^{2+} in the aged rats is likely related to glomerulosclerosis, interstitial fibrosis, and impaired renal plasma flow and solute absorption that has been shown in aged kidneys (Baylis and Schmidt 1996; Choudhury 2004; Zhou et al. 2008). Interestingly, within the aged rats, the renal accumulation of Hg^{2+} was greater in NPX rats than in control rats. This increase may be due to a greater hematological burden of Hg^{2+} , resulting from a lower GFR in these animals. We propose that even though cellular hypertrophic changes do not appear to occur in aged NPX animals, glomerular hyperfiltration likely occurs in the functioning glomeruli and could lead to an increased filtration fraction of Hg^{2+} . This increase may then lead to enhanced uptake of mercuric ions by proximal tubules.

The urinary excretion of Hg^{2+} corresponded to the pattern of renal accumulation of Hg^{2+} in that more Hg^{2+} was excreted in the urine by aged rats than by corresponding young rats. This increase in the urinary excretion of Hg^{2+} in the aged rats is likely due to hyperfiltration in the remaining functional nephrons.

We have shown previously that there is significant axial heterogeneity in the handling of Hg^{2+} along the proximal tubule (Zalups et al. 2014). Indeed, in the present study uninephrectomy had the greatest effect on the disposition of Hg^{2+} in the OSOM. The accumulation of Hg^{2+} in the OSOM was significantly greater in the young NPX rats than in young control rats, suggesting that the compensatory changes associated with uninephrectomy affect the uptake of Hg^{2+} primarily in the OSOM. Previous studies in young rats suggest that enhanced accumulation of Hg^{2+} in the OSOM following uninephrectomy may be due, in part, to the increased expression of specific transporters in the luminal membrane of proximal tubular cells (Zalups 1997; Zalups and Diamond 1987a;

b). Interestingly, the present data demonstrate an increase in the renal expression of System $b^{0,+}$, which has been shown to transport mercuric species (Bridges et al. 2004; Bridges and Zalups 2004) and thus, may account for the increased proximal tubular uptake of Hg^{2+} in the current study. The accumulation of Hg^{2+} in the OSOM was significantly greater in the remnant kidney of aged NPX rats than in aged controls. As discussed previously, this increase may be due to glomerular hyperfiltration.

Uninephrectomy also led to alterations in the hepatic burden of Hg^{2+} , which was significantly greater in young NPX rats than in young control rats. One possible explanation for this finding is that the absence of one kidney increases the circulating levels of Hg^{2+} , which may lead to enhanced hepatic delivery and uptake of Hg^{2+} . The enhanced fecal elimination of Hg^{2+} in the NPX rats correlates with the enhanced hepatic burden. Interestingly, the hematologic burden of Hg^{2+} in the young control rats was similar to that of young NPX rats, which may relate to the increased hepatic uptake of Hg^{2+} from the blood in the NPX animals. The amount of Hg^{2+} excreted in the feces tended to be lower in aged rats than in young rats. This finding is not surprising given that published reports indicate that hepatobiliary excretion of solutes decreases with age (Kroger et al. 1980; Ohta et al. 1988; Schmucker et al. 1985).

The current study is the first to characterize the disposition of Hg^{2+} , a model nephrotoxicant, in an aged model of an acute reduction of renal mass. We show that aging appears to reduce the ability of the remnant kidney to undergo compensatory renal hypertrophy following a 50% reduction in functional renal mass. We also show that aging alters the disposition of Hg^{2+} , particularly in the kidneys. These alterations may make aging kidneys more susceptible to nephrotoxics following an acute, significant reduction of renal mass.

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Highlights

- Aged animals have a reduced ability to undergo compensatory renal hypertrophy
- Aged animals demonstrate greater levels of oxidative stress
- The handling of Hg is altered in aged animals with reduced renal mass

Figure 1.

Outer diameters of proximal tubules from young and aged (control and NPX) rats. The diameters of all the proximal tubules in four, randomly chosen microscopic fields were measured using an eyepiece reticle calibrated with a stage micrometer. Each bar represents the mean $\pm$ standard error of five (control) or seven (NPX) rats. Tubules were measured at a magnification of 400x. *, Significantly different ($p < 0.05$) from the mean of the group of corresponding control rats.

Figure 2.

Real-time PCR analyses of Na⁺, K⁺-ATPase (A), organic anion transporter (Oat1) (B), b^{0,+}AT (C), and vascular endothelial growth factor (Vegf) (D) in kidneys from young control, young NPX, aged control, and aged NPX rats. Each bar represents the mean ± standard error of five (control) or seven (NPX) rats. *, Significantly different (p < 0.05) from the mean of the group of corresponding control rats. +, Significantly different (p < 0.05) from the mean of the group of corresponding young rats.

Figure 3.

Analyses of the formation of 4-hydroxynonenal (HNE) adducts (A) and the expression of glutamate cysteine ligase (Gcl) (B) in kidneys from young control, young NPX, aged control, and aged NPX rats. An HNE Adduct Elisa kit was used to assess the formation of HNE adducts; real time-PCR was used to analyze the expression of Gcl. Each bar represents the mean $\pm$ standard error of five (control) or seven (NPX) rats. *, Significantly different ($p < 0.05$) from the mean of the group of corresponding control rats. +, Significantly different ($p < 0.05$) from the mean of the group of corresponding young rats.

Figure 4.

Analyses of plasma creatinine from young control, young NPX, aged control, and aged NPX rats. Each bar represents the mean $\pm$ standard error of five (control) or seven (NPX) rats. *, Significantly different ($p < 0.05$) from the mean of the group of corresponding control rats. +, Significantly different ($p < 0.05$) from the mean of the group of corresponding young rats.

Figure 5.

Total renal burden expressed as % administered dose g⁻¹ of Hg²⁺ in young and aged rats (control and NPX) injected with 0.5 μmol HgCl₂ kg⁻¹ and euthanized 24 h later. Each bar represents the mean ± standard error of five (control) or seven (NPX) rats. *, Significantly different (p < 0.05) from the mean of the group of corresponding control rats. +, Significantly different (p < 0.05) from the mean of the group of corresponding young rats.

Figure 6.

Concentration (% administered dose g^{-1}) of Hg^{2+} in cortex (A) or outer stripe of outer medulla (OSOM) (B) in young and aged rats (control and NPX) injected with $0.5 \mu\text{mol HgCl}_2 \text{ kg}^{-1}$ and euthanized 24 h later. Each bar represents the mean $\pm$ standard error of five (control) or seven (NPX) rats. *, Significantly different ($p < 0.05$) from the mean of the group of corresponding control rats. +, Significantly different ($p < 0.05$) from the mean of the group of corresponding young rats.

Figure 7.

Urinary excretion (% administered dose) of Hg^{2+} in young and aged rats (Sham and NPX) injected with $0.5 \mu\text{mol HgCl}_2 \text{ kg}^{-1}$ and euthanized 24 h later. Each bar represents the mean $\pm$ standard error of five (control) or seven (NPX) rats. *, Significantly different ($p < 0.05$) from the mean of the group of corresponding control rats. +, Significantly different ($p < 0.05$) from the mean of the group of corresponding young rats.

Figure 8. Hepatic burden (A) and fecal excretion (B) (% administered dose) of Hg^{2+} in young and aged rats (control and NPX) injected with $0.5 \mu\text{mol HgCl}_2 \text{ kg}^{-1}$ and euthanized 24 h later. Each bar represents the mean $\pm$ standard error of five (control) or seven (NPX) rats. *, Significantly different ($p < 0.05$) from the mean of the group of corresponding control rats. +, Significantly different ($p < 0.05$) from the mean of the group of corresponding young rats.

Figure 9.

Hematologic burden (% administered dose) of Hg^{2+} in young and aged rats (Sham and NPX) injected with $0.5 \mu\text{mol HgCl}_2 \text{ kg}^{-1}$ and euthanized 24 h later. Each bar represents the mean $\pm$ standard error of five (control) or seven (NPX) rats. *, Significantly different ($p < 0.05$) from the mean of the group of corresponding control rats. +, Significantly different ($p < 0.05$) from the mean of the group of corresponding young rats.

Table 1

Body weight, weight of left kidney, and weight of total renal mass after nephrectomy for each group of rats. The total renal mass in control animals represents the combined weight of the right and left kidneys. In NPX animals, the total renal mass represents the weight of the left kidney.

	Rat weight (g)	Left kidney weight (g)	Total renal mass (g)
Young – Control	434.56 ± 16.2	1.24 ± 0.04	2.52 ± 0.1
Young – NPX	427.15 ± 43.32	1.78 ± 0.07 ^a	1.78 ± 0.07 ^a
Aged – Control	663.59 ± 0.070	2.05 ± 0.29	4.22 ± 0.63
Aged – NPX	654.73 ± 46.45	2.14 ± 0.19	2.14 ± 0.19 ^a

^a significantly different ($p < 0.05$) from the mean for young control rats.