Experimental Gerontology 75 (2016) 16–23

Contents lists available at ScienceDirect

Experimental Gerontology

journal homepage: www.elsevier.com/locate/expgero

Compensatory renal hypertrophy and the handling of an acute nephrotoxicant in a model of aging

Cláudia S. Oliveira [1], Lucy Joshee, Rudolfs K. Zalups, Christy C. Bridges *

Division of Basic Medical Sciences, Mercer University School of Medicine, Macon, GA, USA

ARTICLE INFO

Article history:
Received 3 November 2015
Received in revised form 17 December 2015
Accepted 2 January 2016
Available online 6 January 2016

Section Editor: Werner Zwerschke

Keywords:
Aging
Kidney
Compensatory hypertrophy
Uninephrectomy
Mercury

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 are 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. 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 et al., 2004; Lopez-Novoa, 2008; Zhou et al., 2008a, 2008b). 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 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 nephrotoxicants. Aged individuals may be exposed to various nephrotoxicants 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 nephrotoxicants 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 nephrotoxicants in the early stages of CKD. This continued exposure may enhance morbidity and even mortality.

* Corresponding author at: Mercer University School of Medicine, Division of Basic Medical Sciences, 1550 College St., Macon, GA 31207, USA.
E-mail address: Bridges_cc@mercer.edu (C.C. Bridges).
[1] Current address: Post-Graduate Course in Biological Science — Toxicological Biochemistry, Federal University of Santa Maria, Santa Maria, RS, Brazil.

http://dx.doi.org/10.1016/j.exger.2016.01.001
0531-5565/

Owing to the growing population of aged individuals, the incidence of diabetes and hypertension in this population, and the prevalence of nephrotoxicants 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 nephrotoxicants.

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. 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 $HgCl_2$ according to our previously published protocol (Bridges et al., 2008a, 2008b). The injection solution contained radioactive mercury ($[^{203}Hg^{2+}]$) and was designed to deliver 1 μCi $[^{203}Hg^{2+}]$ to each animal. $[^{203}Hg^{2+}]$ 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 $HgCl_2$ 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. 24 h after injection with $HgCl_2$, animals were euthanized and organs/tissues were harvested.

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.

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 Hg^{2+} content. A separate sample of blood was placed in a Microtainer plasma separation tube in order to estimate the content of Hg^{2+} in the plasma and cellular fractions. The total volume of blood was estimated to be 6% of body weight (Lee and Blaufox, 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-traverse 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-h 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-h experiment were counted for estimation of Hg^{2+} content.

The content of $[^{203}Hg^{2+}]$ 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