Review Article

Is there a role for oxidative stress and mitochondrial dysfunction in age-associated bladder disorders?

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ABSTRACT

Millions of individuals worldwide are affected by age-related lower urinary tract symptoms (LUTSs), including impaired detrusor contractility, detrusor overactivity, decreased bladder sensation, as well as increased bladder capacity often resulting in incomplete bladder emptying. Yet, the underlying factors that contribute to these symptoms are not known and there are few therapies to treat these disorders. Because of the complex pathophysiology, a number of animal models have been studied over the years to better understand mechanisms underlying patient symptoms. Such animal models can aid in the investigation of aspects of age-associated LUTSs that cannot be pursued in humans as well as to develop and test potential therapies. In addition, the search for urinary factors that may be a causative agent has resulted in the discovery of a number of potential targets that could serve as predictive biomarkers which can aid in early diagnosis and treatment of these chronic disorders. Recent evidence has supported a role for chronic changes in mitochondrial function and oxidative stress (along with production of reactive oxygen species) and abnormal urodynamic behavior in older patients. This review discusses new insights into how aging alters fundamental cellular processes that impair signaling in the bladder wall, resulting in abnormal voiding function.

KEYWORDS: *Bladder, Mitochondria, Pathophysiology*

INTRODUCTION

Aging has been defined as the continued loss of homeostatic reserve. It is a complex, biological process controlled by multiple genetic, epigenetic, and environmental factors that result in progressive stress to the cell, tissue, or organ in question [1]. Aging-related bladder dysfunction and lower urinary tract symptoms (LUTSs) represent an increasing problem in developed countries due to increased life expectancy [2,3]. LUTSs are generally divided into storage (irritative), voiding (obstructive), and postmicturition components. Storage symptoms include urgency, frequency, nocturia, and urgency incontinence (i.e., the overactive bladder syndrome). Voiding symptoms comprise reduced force of stream, hesitancy, inability to empty the bladder, and straining. Postmicturition symptoms include feeling of incomplete emptying and postmicturition dribble. Most of these symptoms have been suggested to be age dependent and attributed to various factors including reduced bladder capacity, changes in bladder sensation, and on urodynamic investigation, detrusor overactivity (DO). However, the pathophysiology behind the dysfunctions is sometimes difficult to establish since what can be attributed to "normal aging" cannot be separated from what is caused by comorbidities. LUTS is an ever-increasing problem: an estimated 45% of the 2008 worldwide population (4.3 billion) was affected by at least one LUTS, reducing the quality of life and this number is expected to significantly increase over time [4].

ANIMAL MODELS OF AGING-RELATED BLADDER DYSFUNCTION

Animal models allow detailed investigation of structural and functional aspects of the micturition pathways and changes occurring with aging. In addition, the genetically modified mouse models allow further understanding and targeting of specific genes. The influence of aging on bladder structure and function has been studied in *in vivo* and/or *in vitro* studies performed mostly in rodents of different strains and/or gender. These include C57Bl6 mice, the senescence-accelerated prone mice (SAMP8), Fisher 344 rats, and many others [5-10]. The relation between aging *per se* and external influences on the

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detrusor from diseases in the nervous system, in the vascular supply, and in the lower urinary tract smooth muscles is poorly understood in humans. In animals, kept under constant laboratory conditions, theoretically, the influence of external influences can be reduced, which should enable the study of the effect of age only on bladder function. However, this does not seem to provide consistent results in part due to differences between species, gender, or strain. Cystometry has yielded somewhat more variable results mainly due to species and/or gender differences and/or anesthesia [11,12]. Ischemia, which is a main risk factor in aging [13,14], has shown to result in dynamic changes, resembling in the initial phase DO (e.g., increased nonvoiding contractions, increased voiding frequency, and decreased voided volume), and progressing with time to detrusor underactivity (e.g., decreased voiding frequency). Thus, depending on the underlying risk factors, aging may have variable effects on bladder function. Data from animal models, which seem to be as variable as the data from human studies in different clinical conditions, may be useful for understanding the progression of bladder function with age.

Aging is associated with mitochondrial dysfunction and increased oxidative stress

At the cellular level, mitochondria are considered major players in energy production, intracellular communication, and are associated with a number of age-related diseases [15-18]. Mitochondria, considered the powerhouse of organelles and generate 95% of all cellular energy, play a key role in cellular homeostasis, including generation of reactive oxygen species (ROS), apoptosis, regulation of intracellular calcium, and generation of ATP via oxidative phosphorylation and release of factors that modulate pro- and antiaging signaling pathways. Dysfunctions in mitochondrial metabolic capacity and structural alterations (i.e., accumulation of damaged mitochondria and enhanced cross-linking of proteins) can contribute to oxidative stress and cell death during the aging process.

Oxidative stress is broadly defined as a disturbance in a pro-oxidant–antioxidant balance (i.e., uncontrolled increases in the production of reactive oxygen [or nitrogen] species or deficiencies in antioxidant defense mechanisms), which can lead to potential damage. Oxidative metabolism can yield free radicals and other unstable oxygen- and nitrogen-containing molecules [19-22]. When produced at low or physiological levels, ROS can regulate a number of processes including maintenance of vascular tone and signal transduction. However, at higher levels, excessive ROS can result in oxidative damage to lipids, proteins, carbohydrates, and DNA, leading to the generation of secondary reactive species and finally loss of function and cell death [19-22]. ROS (and reactive nitrogen species, RNS) are also generated during radiation therapy, and in the bladder, radiation toxicity generates LUTS [23,24]. Sources of ROS can include nitric oxide synthase, xanthine oxidase, as well as the mitochondria, an essential supplier of energy. Mitochondria have been described as both a primary source and also target of ROS. ROS is a general term that includes a number of species such as the superoxide anion, which is often increased in conditions of ischemia or hypoxia. Excessive amounts of superoxide can interact with nitric oxide to form peroxynitrite which is a pro-oxidant capable of rapidly diffusing to nearby cells inducing damage. The highly reactive hydroxyl radical is thought to mediate most free radical-induced tissue damage [19-22].

Mitochondrial DNA (mtDNA) is more susceptible to oxidative damage than nuclear DNA due in part to proximity of mtDNA to the respiratory chain and decreased availability of repair mechanisms [25]. Damage to mtDNA can not only result in mitochondrial dysfunction but also trigger inflammatory and innate immune responses [26,27]. Studies suggest that oxidative stress also plays a role in fibrotic diseases by augmenting the production of various regulators of fibrosis such as growth factors, angiogenic factors, and cytokines. In the airways, augmented ROS is involved in increased vascular permeability and bronchial hyperresponsiveness, characteristic features of asthma [28,29]. Because mitochondria are the major consumers of cellular oxygen, it is not surprising that these organelles are significantly impacted by hypoxia and ischemia. Reduced levels of oxygen result in augmented ROS production, decreases energy production and changes in mitochondrial morphology.

EVALUATION OF AGE-ASSOCIATED CHANGES IN LUT FORM AND FUNCTION

Aging is associated with an impairment of blood vessel function and changes may occur in the vasculature on the molecular, cellular, structural, and functional levels [14]. Endothelial dysfunction leads to oxidative stress and increased levels of pro-inflammatory cytokines, which represents an independent risk factor for the development of atherosclerosis and hypertension. Evidence from epidemiologic, clinical, and animal basic research suggests that aging-associated changes in the pelvic vasculature, resulting in atherosclerosis and vascular dysfunction, may be important factors in the generation of LUTS [30,31]. Evidence from clinical and basic research suggests that atherosclerosis in both genders can induce a reduction of bladder blood flow, leading to chronic ischemia. Chronic bladder ischemia and repeated ischemia/reperfusion during a micturition cycle may produce oxidative stress and lead to denervation of the bladder and the expression of tissue damaging molecules in the bladder wall [32,33]. Studies in animal models suggest that the extent of bladder dysfunction in chronic ischemia depends on the degree and duration of ischemia. This appears to be responsible for the development of DO progressing to underactivity and inability to empty the bladder [34]. When bladder ischemia becomes severe and prolonged, progression of denervation and damage to detrusor muscle with fibrosis formation may cause voiding symptoms.

Further, age-related changes in the extracellular matrix (ECM) may also impact the function of tissues in the bladder wall. Despite having different etiologies, most chronic fibrotic disorders produce a persistent production of similar factors including ROS that stimulate ECM production, which progressively destroys the organ's architecture and, in turn, its function [35,36]. Mitochondria are the primary source of ROS; pathologies associated with mitochondrial dysregulation

(including aging) lead to overproduction of ROS, superoxide, and factors that promote fibrosis [37]. As the bladder fills, the coordinated recruitment of collagen fibers across both the smooth muscle and lamina propria layers, essential for the elasticity of the bladder wall, is lost during aging [38]. Further, this impacts the ability of the urothelium to "sense" changes in mechanical deformation that occurs during a micturition cycle and release mediators that may influence sensation.

Much less is known about the effect of aging on urothelial changes. The urothelium, which lines the inner surface of the renal pelvis, ureters, and urinary bladder, not only forms a high-resistance barrier to ion, solute and water flux, and pathogens, but also functions as an integral part of a "sensory web" which receives, amplifies, and transmits information about its external milieu [39,40]. Structural studies have shown urothelial thinning, granular appearance of the umbrella cell layer often containing what appears to be cellular debris in all layers. These could be the result of oxidative stress and altered mitochondrial dysfunction. In support, increased ROS in cultured urothelial cells, associated with upregulation of transient receptor potential cation channel subfamily M member 8, decreased total antioxidant capacity, and significantly increased levels of lipid peroxides, malondialdehyde, and inducible nitric oxide synthase, all markers of oxidative stress, as well as ultrastructural alterations in mitochondria with accumulation of lipofuscin have been reported [41,42]. Further, recent studies have revealed an age-related decrease in lysosomal function in urothelium, which may have significant effects on the physiological function of the bladder [43]. Lysosomal dysfunction is associated with a number of age-related pathologies that can affect all organ systems [44,45]. Lysosomes perform a complex array of functions including promoting the turnover of cellular organelles and proteins and regulation of various activities such as plasma membrane repair [44,45]. A dysfunction in lysosomal system can have debilitating effects on cellular function as is observed in age-related neurodegenerative diseases including Alzheimer's and Parkinson's. Recent findings showing that lysosomal function is diminished in aging demonstrate that aged (urothelial) cells exhibit a gradual accumulation of metabolic waste products and cellular debris [43]. Defects in this function may alter the homeostatic chemical balance of the urothelium and cellular communication with underlying layers. This in turn could lead to altered detrusor function, manifesting in the various clinical conditions that are observed in the elderly.

CONCLUSIONS AND FUTURE DIRECTIONS

While no animal model can be expected to reproduce all the various symptoms experienced by humans, more complex models are needed to mimic the symptoms and systemic changes found in aged patients which include incontinence, overactivity, and/or the inability to empty. Because all aspects of the disease may not be readily addressed by a single animal model, several models may be required, to create a reasonable picture of both pathophysiology and the time course of the disease (which includes temporal changes in biomarkers). While the underlying mechanisms are likely to be complex, they may be controlled in part by multiple genetic, epigenetic,

and environmental factors. Further studies are needed to correlate findings in animal models to patient symptoms to provide better insights and new strategies for the clinical management of these bladder disorders. In addition, future translational studies should also consider how changes in bioenergetics and oxidative stress impact bladder aging to develop new therapeutic strategies that may be an important tool to treat age-related bladder control problems.

Financial support and sponsorship

Conflicts of interest

There are no conflicts of interest.

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