F_2-Isoprostanes as a Biomarker of Oxidative Stress in the Mouse Bladder

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Purpose: We theorized that progressive bladder dysfunction due to clinical diagnoses such as outlet obstruction occurs as a result of cyclical oxidative stress events. We hypothesized that measurement of F_2-isoprostane, a marker of lipid peroxidation, could serve as a biomarker of oxidative stress in the murine bladder.

Materials and Methods: At age 5 to 6 weeks oophorectomized female mice were subjected to 1 of 2 bladder injury models, that is partial bladder outlet obstruction or acute bladder distension. The time points studied after injury included 4, 8 and 16 weeks after obstruction, and 0 to 48 hours after acute bladder distension. In a separate group short-term repetitive acute bladder distension was performed every other day for 14 days. Bladder samples were analyzed for F_2-isoprostane using gas chromatography and mass spectroscopy. Mean tissue F_2-isoprostane levels were compared.

Results: F_2-isoprostane increased significantly after 4 weeks of partial bladder outlet obstruction from 1.46 ng/gm in controls to 2.31 ng/gm at 4 weeks ($p = 0.01$). Eight and 16 weeks after partial bladder outlet obstruction F_2-isoprostane remained significantly elevated (2.39 and 2.48 ng/gm, respectively). Acute bladder distension resulted in a significant increase in F_2-isoprostane immediately after distension compared to controls (1.6 vs 0.75 ng/gm, $p = 0.04$). In mice that underwent repetitive acute bladder distension F_2-isoprostane did not change.

Conclusions: Measurement of tissue F_2-isoprostane in the bladder reflects the progression of oxidative stress, primarily in chronic injury models such as partial bladder outlet obstruction. The usefulness of F_2-isoprostane measurements in shorter term injury models requires further study.

Key Words: urinary bladder, oxidative stress, urinary bladder neck obstruction, F_2-isoprostanes, mice

Abbreviations and Acronyms

BD = bladder distension

F_2-IsoP = F_2-isoprostane

pBOO = partial bladder outlet obstruction

ROS = reactive oxygen species

T = time point

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Study received Vanderbilt University Medical Center institutional animal care and use committee approval.

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For several years our research group has been interested in the critical role of hypoxia in the development of progressive bladder dysfunction. Specifically, using 2 unique murine models of bladder injury (pBOO and BD) we previously reported that hypoxic pathways are up-regulated after experimental injury.[1,2] Using similar models our group and others

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theorized that bladder injury due to these types of insult is mediated by oxidative stress and free radicals.[3,4]

Oxidative stress represents an imbalance between ROS production and innate cellular detoxifying mechanisms.[5] F_2-IsoPs are compounds structurally similar to prostaglandins that have garnered significant interest in various fields as a biomarker of in vivo oxidative stress. F_2-IsoPs have been extensively studied in a number of chronic disease states, including aging, diabetes, metabolic syndrome, atherosclerosis and cancer.[6,7]

Our group was interested in better methods of quantifying chronic hypoxic injury in the mouse bladder. We hypothesized that F_2-IsoPs could serve as a metric of oxidative stress at the tissue level in experimental models of murine bladder injury. We describe our initial experience with in vivo F_2-IsoP characterization in the mouse bladder.

MATERIALS AND METHODS

The Vanderbilt University Medical Center institutional animal care and use committee approved all animal studies.

General Mouse Procedures

All murine experiments were performed in C57Bl/6 females after bilateral oophorectomy at age 5 to 6 weeks because estrogen may blunt the inflammatory process in the murine bladder.[8] One week after oophorectomy the mice were used in the separate experiments described.

Partial Bladder Outlet Obstruction

With the mouse under isoflurane anesthesia pBOO was created as previously reported.[1,9] Mice were aged after injury for various intervals, including 4, 8 and 16 weeks. Oophorectomized female mice served as controls in initial experiments while age matched oophorectomized female controls were used as indicated.

Bladder Distension

BD was performed as previously reported.[2] Briefly, using isoflurane anesthesia the mouse urethra was catheterized in sterile fashion with a 24 gauge angiocatheter and connected to a gravity dependent water column filled with sterile 0.9% saline warmed to 37C. The water columns were filled to maintain a constant 60 cm H_2O pressure for 90 minutes, after which the bladder was drained.

A time course of single acute BD experiments was performed. Bladders were harvested after a single BD at certain points, including T0—immediately after BD, T0.5—at 30 minutes of recovery, T1—at 60 minutes, T3—at 3 hours, T24—at 24 hours and T48—at 48 hours. In a separate experimental group BD was performed once daily in repeat fashion every other day for 14 days. Controls for acute and repetitive experiments consisted of female mice that underwent catheterization and 90 minutes of anesthesia without BD.

F_2-IsoP Measurement

Bladder and kidney tissues were snap frozen in liquid nitrogen and stored at −80C until analysis at the Vanderbilt University Eicosanoid Core Laboratory. Samples were analyzed by gas chromatography and mass spectroscopy. Milne et al previously reported a detailed protocol for this analysis.[10] F_2-IsoP quantity is reported in ng F_2-IsoP/gm tissue. Levels are shown as the mean ± SD.

Statistical Analysis

Group means were compared with nonparametric tests, including the Mann-Whitney U and Kruskal-Wallis tests. Nonparametric correlation coefficients were determined using the Spearman test. Tests were calculated using Prism® 5.0 with $p \leq 0.05$ considered significant.

RESULTS

F_2-Isoprostane Levels Increased Proportionally with pBOO Duration

In surgically obstructed mouse bladders the duration of pBOO was proportional to F_2-IsoP levels compared to those in nonoperated controls (fig. 1, *A*). Spearman coefficient analysis revealed a positive correlation between pBOO duration and the tissue F_2-IsoP level (r = 0.72, 95% CI 0.5367−0.8404,

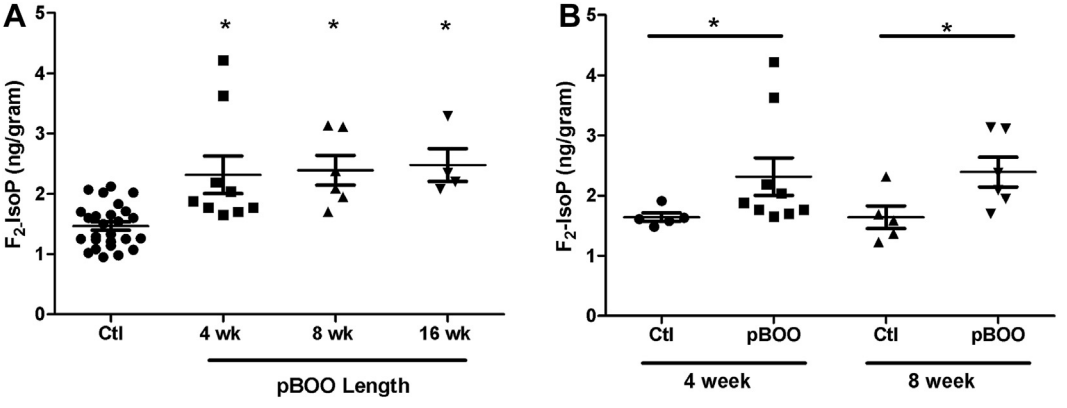

Figure 1. Tissue F_2-IsoP after pBOO. *A*, control (*Ctl*) vs 4, 8 and 16-week pBOO groups. *B*, age matched controls, and experimental 4 and 8-week groups. Error bars represent ± SD. Horizontal lines represent mean. Asterisk indicates $p < 0.05$.

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