A Murine Model of Inflammatory Bladder Disease: Cathelicidin Peptide Induced Bladder Inflammation and Treatment With Sulfated Polysaccharides

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Abbreviations and Acronyms

GAG = glycosaminoglycan

IC = interstitial cystitis

MPO = myeloperoxidase

PMN = polymorphonuclear leukocyte

SAGE = semi-synthetic GAG ether

t = time

Study received University of Utah institutional animal care and use committee approval.

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Purpose: Studies show that LL-37 is a naturally occurring urinary defensin peptide that is up-regulated during urinary tract infections. Although normal urinary LL-37 levels are antimicrobial, we propose that increased LL-37 may trigger bladder inflammation. We further suggest that anti-inflammatory sulfated polysaccharides known as semi-synthetic glycosaminoglycan ether compounds can treat/prevent LL-37 mediated bladder inflammation.

Materials and Methods: C57BL/6 mice were catheterized/instilled with LL-37 (320 μM, 150 μl) for 45 minutes. Animals were sacrificed at 12 and 24 hours, and tissues were examined using hematoxylin and eosin. Separate experiments were performed for myeloperoxidase to quantify inflammation. GM-1111 semi-synthetic glycosaminoglycan ether treatments involved instillation of 10 mg/ml for 45 minutes directly before or after LL-37. Tissues were harvested at 24 hours. To compare semi-synthetic glycosaminoglycan ether efficacy, experiments were performed using 10 mg/ml heparin. Finally, tissue localization of semi-synthetic glycosaminoglycan ether was examined using a fluorescent GM-1111-Alexa Fluor® 633 conjugate.

Results: Profound bladder inflammation developed after LL-37. Greater tissue inflammation occurred after 24 hours compared to that at 12 hours. Myeloperoxidase assays revealed a 21 and 61-fold increase at 12 and 24 hours, respectively. Semi-synthetic glycosaminoglycan ether treatment after LL-37 showed mild attenuation of inflammation with myeloperoxidase 2.5-fold below that of untreated bladders. Semi-synthetic glycosaminoglycan ether treatment before LL-37 demonstrated almost complete attenuation of inflammation. Myeloperoxidase results mirrored those in controls. In heparin treated bladders minimal attenuation of inflammation occurred. Finally, instillation of GM-1111-Alexa Fluor 633 revealed urothelial coating, significant tissue penetration and binding to endovasculature.

Conclusions: We developed what is to our knowledge a new model of inflammatory bladder disease by challenge with the naturally occurring urinary peptide LL-37. We also noted that a new class of anti-inflammatory sulfated polysaccharides prevents and mitigates bladder inflammation.

Key Words: urinary bladder, inflammation, glycosaminoglycans, rosacea, mice

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INFLAMMATORY conditions of the bladder are a significant health concern. Neurogenic bladder disease can result from excess inflammation, leading to fibrosis. In children many diseases cause neurogenic bladder disease, including myelomeningocele/spina bifida. These processes overlap in adults, in whom chronic inflammatory bladder disorders such as IC result in debilitating urinary symptoms. More than 4 million people in the United States have IC and the cost/disease burden exceeds $750 million dollars annually.[1] Currently IC treatment has been suboptimal due to its uncertain cause and pathogenesis.[2–5]

We describe the development of a reproducible mouse model of bladder inflammation. The model builds on studies of epithelial cells in the human skin inflammatory disorder, rosacea. Individuals with rosacea express abnormally high epithelial skin levels of the cationic antimicrobial peptide cathelicidin and its post-enzymatic cleaved peptide product LL-37,[6,7] resulting in profound skin inflammation. LL-37, a host defense peptide, is produced from the C-terminus of the hCAP18 precursor protein and made by a multivariate cell population, including epithelial cells and circulating neutrophils.[8] Human and mouse urothelial cells naturally produce LL-37.[9] Also, during pediatric urinary tract infection (pyelonephritis and/or cystitis) urinary LL-37 is significantly increased.[9] We have preliminary data on increased levels of urinary LL-37 in noninfected children with spina bifida. LL-37 also contains immunomodulatory properties that trigger inflammation.[7,8] Furthermore, low concentrations of LL-37 (13 to 25 μM) can be cytotoxic against eukaryotic cells.[10]

We propose that increased urinary LL-37 triggers inflammatory cascades, contributing to bladder inflammation and possibly fibrosis. Numerous insults, including urinary tract infections and toxic cationic urinary metabolites, could cause injury to the protective urinary GAG layer and damage the urothelium. Consequently urothelial permeability and inflammatory cascade activation increase.

In this study we also examined a new family of anionic, partially lipophilic polysaccharide derivatives known as SAGEs. These anionic polymers mimic sulfated native urothelial GAGs. As with heparin and partially desulfated heparin,[11] SAGEs inhibit PMN proteases, and P and L-selectin mediated influx of leukocytes into areas of inflammation (Zhang et al, unpublished data). SAGEs also show saturable, high affinity binding to LL-37, thereby modulating the interaction with its natural targets (Zhang et al, unpublished data). We tested the hypothesis that SAGEs attenuate bladder inflammation by 1 or more modes of action, including remediation of damage to the GAG layer, charge neutralization of toxic cationic metabolites, vascular stabilization and modulation of inflammatory cell activity.

MATERIALS AND METHODS

Bladder Inflammation

Experiments were performed in accordance with the institutional animal care and use committee at our university. We used 8 to 12-week old female C57BL/6 mice. LL-37 was obtained in high performance liquid chromatography-homogenous form (peptide sequence: LLGDFFRKSKEKIGKEFKRIVQRIKDFLRNLVPRTES) and dissolved in nanopure water to yield 320 μM. Each group consisted of 6 mice. After establishing isoflurane anesthesia a 1.5 cm silicone catheter with an inner and outer diameter of 0.30 and 0.64 mm, respectively, was introduced transurethrally. After complete urine drainage 150 μl pyrogen-free 0.9% sodium chloride were instilled for 1 minute and emptied. LL-37 (320 μM) was instilled at a volume equal to capacity (150 μl),[12–16] as previously described (intravesical t = 45 minutes). Controls consisted of pyrogen-free 0.9% sodium chloride instillation. Substances were infused slowly to avoid trauma and vesicoureteral reflux.[12–16] The instillation syringe was kept on the catheter to ensure no solution leakage.[12–16] At 45 minutes the bladders were emptied to completion. Depending on the experimental group the animals were sacrificed at 12 or 24 hours.

SAGE GM-1111 and Heparin Treatment

We examined SAGE and heparin (each pH 7.0) treatment in 2 LL-37 induced bladder inflammation groups. In the 4 group 1 mice we first instilled LL-37 (320 μM at 150 μl) for 45 minutes and then emptied it. Immediately thereafter SAGE or heparin (10 mg/ml at 150 μl) was instilled for 45 minutes and then emptied. Bladders were harvested at 24 hours. In the 4 group 2 mice we first instilled SAGE or heparin (10 mg/ml at 150 μl) for 45 minutes, emptied the bladders and challenged them with LL-37 (320 μM) for 45 minutes. Tissues were harvested at 24 hours. All bladders were hemisected, processed and stained with hematoxylin and eosin, or tissue MPO assays were done. Statistical analysis of MPO data on SAGE treated samples was performed with the unequal variance 2-tailed Student t test with $p < 0.05$ considered statistically significant.

Bladder Coating

We synthesized SAGE GM-1111-Alex Fluor 633 bioconjugate as described in another series (Zhang et al, unpublished data). Bladders were instilled with GM-1111-Alexa Fluor 633 bioconjugate (10 mg/ml at 150 μl) for 45 minutes and harvested immediately thereafter (t = 0) or at 24 hours (t = 24). Tissue sections were counterstained with 4,6-diamidino-2-phenylindole. Fluorescence imaging was done with a FV1000 Confocal IX81 microscope (Olympus®).

Tissue

Collection and histological evaluation. Bladders were removed and split longitudinally. One section was fixed