



#521-Increased urinary bladder susceptibility to infections through modulation of urothelial NADPH oxidase (Nox)-associated oxidative stress in a canine model of lower spinal cord injury

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ABSTRACT

The urothelium contributes to bladder function by communicating with the central nervous system via afferent nerves. Acute injury, such as, spinal cord transection results in altered bladder urothelial barrier function, modification of the urothelium and/or loss of epithelial integrity^{1,2,3}.

We determined the effect of progressively more extensive decentralization surgeries on the frequency of urinary tract infections (UTIs) and the role of urothelial reactive oxygen species (ROS)-generating NADPH oxidase (Nox) enzymes .

METHODS

Decentralization surgeries:

- **Group 1** (N=6): all dorsal and ventral sacral spinal roots and the hypogastric nerves
- **Group 2** (N=7): all dorsal and ventral sacral spinal roots, the L7 dorsal roots and hypogastric nerves
- **Group 3** (N=8): all dorsal and ventral sacral spinal roots, the dorsal roots of L6 and L7 and the hypogastric nerves.

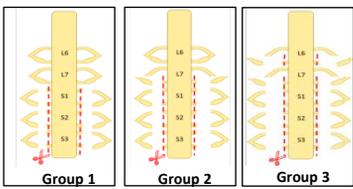


Figure 1. Illustrations of surgical decentralization procedure. S=sacral; L=lumbar

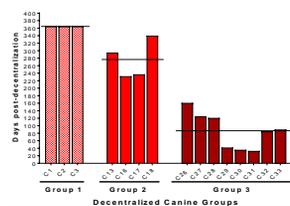


Figure 2. Days post-surgery in the 3 canine groups. C=canine

- Hematoxylin and eosin stain was used to assess tissue histopathology
- TUNEL assay was used to evaluate apoptotic rate
- NADPH-dependent superoxide production was quantitated with lucigenin enhanced chemiluminescence. Tiron, a superoxide scavenger, was used to verify the detection of superoxide.
- Dihydroethidium (DHE) fluorescence was used to detect intracellular superoxide generation in bladder tissue

RESULTS

Parameter	Group 1	Group 2	Group 3
Decentralized Canine Groups			
# of animals/group	6	7	8
# of animals having UTIs/group % of total	None	4 57%	6 75%

Table 1. Loss of bladder afferent innervation in canines creates recurrence of UTIs in the decentralized canine groups

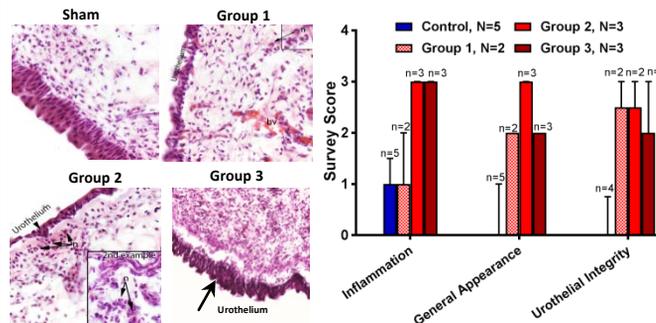


Figure 3. Increased signs of inflammation in bladder tissue of decentralized canines. n=neutrophil; bv=blood vessel

Figure 4. H&E Scoring

Table 2. H&E assessment

Parameter	Score	0	1	2	3
Inflammation	None	Mild neutrophilic and/or lymphocytic infiltration	Moderate neutrophilic and/or lymphocytic infiltration	Severe neutrophilic and/or lymphocytic infiltration	
General Appearance	Normal	Mild change in vascularity and/or change in cellularity	Moderate increase in vascularity and/or change in cellularity	Severe increase in vascularity and/or change in cellularity	
Urothelial Integrity	Normal	Mild change from normal	Gross change in density, but generally intact	Thin and detached	

RESULTS

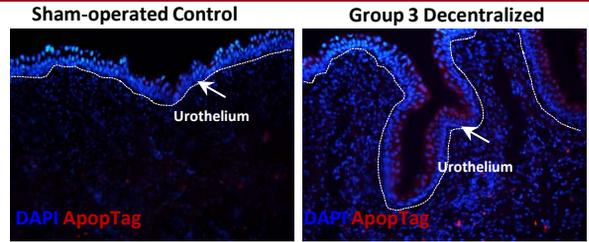


Figure 5. Fluorochrome-based TUNEL assay shows increased rate of apoptosis in the decentralized bladder urothelium. The white dotted line indicates the boundary between the urothelium and suburothelium.

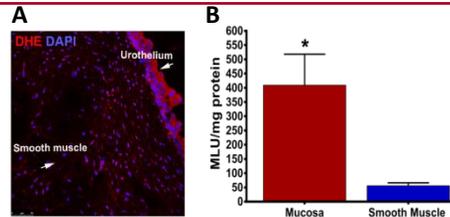


Figure 6. Superoxide levels in Normal canine bladder. **A.** Image of full thickness bladder tissue stained with DHE (in red) and DAPI stained nuclei (in blue) revealed highest density of superoxide in the urothelium. **B.** Lucigenin of bladder mucosa and smooth muscle lysates showed higher superoxide levels in mucosa compared to smooth muscle. MLU = mean light units.

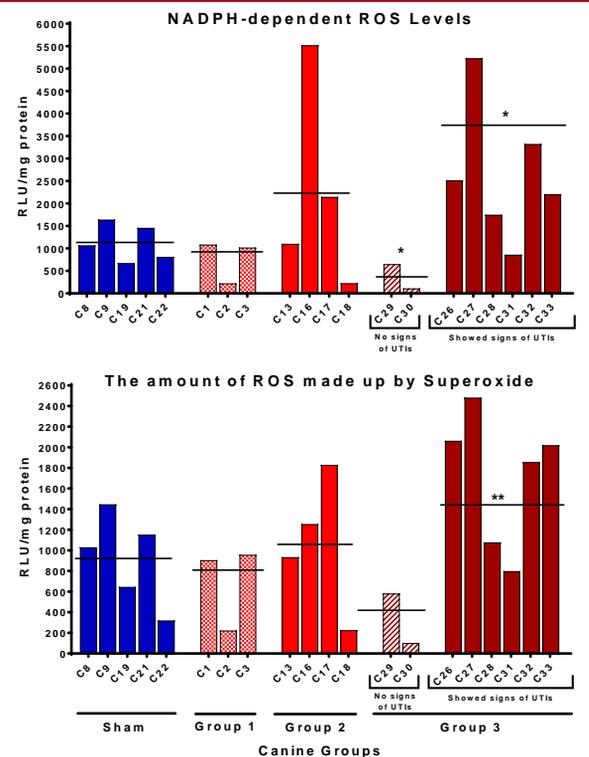


Figure 7. **A.** Lucigenin data show total NADPH-dependent ROS levels in group 1 were comparable to shams, decreased in one animal in group 2 with long-term decentralization and recurrent UTIs and increased in 60% of group 3 canines that showed signs of UTIs. **B.** Superoxide levels were increased in 60% of group 3 canines with UTIs. *:p<0.05, **:p<0.01.

CONCLUSIONS

- Progressively increased bladder de-afferentation predisposes to UTIs.
- The mechanism may involve Nox-driven superoxide redox processes.
- Infection induced inflammation increase ROS production in the bladder mucosa.
- Sympathetic bladder innervation has no apparent effect on susceptibility to UTIs.

REFERENCES

1- De Groat WC. et al. *Compr Physiol*. 2015; 5(1): 327–396.
 2- Birder LA. et al. *Prog Brain Res*. 2006;152:135–46.
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