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N(G)-NITRO-L- ARGININE METHY ESTER (L-NAME), A NITRIC OXIDE SYNTHASE INHIBITOR, DIMINISHES OXIDATIVE DAMAGE IN RABBIT URINARY BLADDER SMOOTH MUSCLES FOLLOWING PARTIAL OUTLET OBSTRUCTION

Hypothesis / aims of study

We have evidence that reactive nitrogen species (RNS) mediates damage to the urinary bladder subjected to partial outlet obstruction (POO). Nitric oxide (NO) can react with superoxide anions (O2-), yielding the toxic oxidizing agent, peroxynitrite (ONOO-). Peroxynitrite induces nitration of tyrosine residues, lipid peroxidation in biologic membranes and oxidation of proteins leading to changes in structure and function and alterations in signaling pathways. In the present study we investigated the effects of N (G)-nitro-L- arginine methy ester (L-NAME) on the oxidative damage induced by partial outlet obstruction.

Study design, materials and methods

32 male New Zealand White rabbits were divided into 8 groups of 4 rabbits each. Group 1 served as controls. Group 2 underwent sham surgeries. Group 3, 5 and 7 underwent partial outlet obstruction for 1 day, 3 days and 7 days respectively. Groups 4, 6 and 8 were given L-NAME (6mg/ kg body weight, intraperitoneally per day) beginning one week prior to obstruction and continuing throughout the experiment. They were then obstructed for 1day, 3 days and 7 days. Bladders were excised and weighed. Full thickness sections were taken and fixed in buffered formalin for immunohistochemistry and the balance of the bladder was separated between muscle and mucosa, frozen and stored at -70^{o}C for molecular analyses. The effect of POO on nitrotyrosine content was evaluated using western blotting techniques. Tissue sections from all groups were immunostained with anti- nitrotyrosine antibody to see the distribution of nitrotyrosine. In addition, nerve density was quantitated by neurofilament immunohistochemistry and image analysis.

Results

Nitrotyrosine was present in all bladders**.** There was a twofold increase in the expression of nitrotyrosine in 7 days obstructed rabbit bladders when compared to control, 1 day and 3 days of obstruction. The L-NAME treated groups showed significantly lower expression of nitrotyrosine. Immunohistochemistry confirmed the increased expression of nitrotyrosine in obstructed bladders, and also confirmed the downregulation of nitrotyrosine in L-NAME treated rabbit bladders (Fig.1). Interestingly, the nerve count progressively declined over the 7day period. At each time point, the nerve density of the L-NAME treated groups were significantly higher than their non-treated counterparts. (Fig.2).

Fig. 1

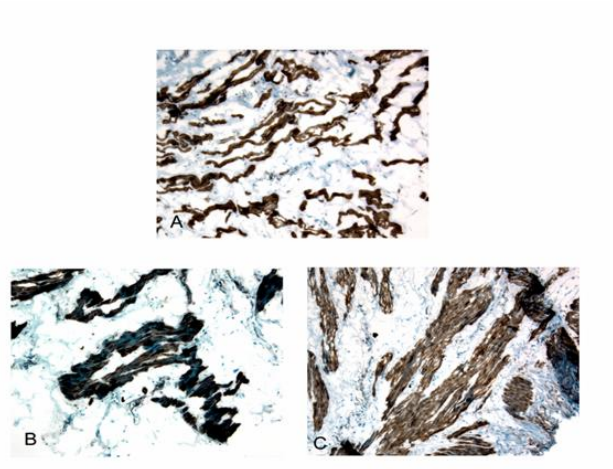
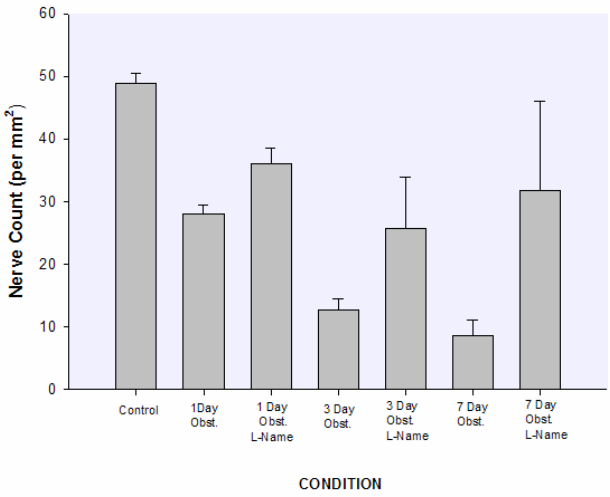


Figure : Rabbit bladder immunostained with Nitrotyrosine (A) control with diffuse staining throughout detrusor smooth muscle.100x (B) 7 day obstructed with very dark staining showing significant increase of nitrotyrosine. 100x (C) Lname treated 7 day obstructed staining similar in density to the control demonstraiting no increase in nitrotyrosine. 100x

Fig. 2



Interpretation of results

The presence of nitrotyrosine in the POO rabbit bladders indicates that oxidants derived from nitric oxide such as peroxynitrite are generated during bladder outlet obstruction and may be involved in its pathogenesis. All L-NAME treated groups showed significant downregulation in the expression of nitrotyrosine. Since peroxynitrite is generated from super oxide and NO, inhibition of NO synthesis is expected to reduce peroxynitrite formation.Since nerves have been shown to be the most sensitive organelles of the bladder to obstructive dysfunction, the protective effect of L-NAME indicates that nitrotyrosine generation in the obstructed bladders may play a key role in the nerve damage observed.

Concluding message

POO clearly resulted in an increased expression of nitrotyrosine and progressive denervation. Pretreatment with L-NAME significantly inhibited the generation of nitrotyrosine and resulted in a significant preservation in nerve density, which indicates that L-NAME reduces the free radical damage associated with POO.

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