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# MOLECULAR MECHANISM OF BLADDER ANGIOGENIC REACTIONS TO CHRONIC ISCHEMIA

**Aims of Study**

Chronic ischemia has been shown to alter bladder structure and function. Our aim was to examine how the bladder vasculature reacts to chronic exposure to ischemia. We studied the anatomical changes of rabbit bladder structure and vascularity in relationship to expression of vascular endothelial growth factor (VEGF), VEGF receptors, and transforming growth factor beta (TGF-beta) at varying durations of atherosclerosis-induced bladder ischemia.

**Methods**

New Zealand White Rabbits were divided into chronic bladder ischemia (CBI, n=15) and age-matched control (n=15) groups. The CBI group underwent balloon endothelial injury of the iliac arteries and received a 0.5% cholesterol diet for 4 weeks. Iliac artery blood flow and bladder blood flow were recorded at 8, 12 and 16 weeks after the balloon injury. Animals were sacrificed at these time points and the bladder tissues were processed for histologic and immunohistochemical staining and RNA isolation. Bladder vascular density was analyzed by immunostaining with anti-CD31 primary antibody. Tissue content of VEGF was determined by enzyme immunoassay (EIA). Gene expression of VEGF, and its receptors were determined by semiquantitative RT- PCR analysis using primers for VEGF, VEGFR1 and VEGFR2.

**Results**

Arterial balloon injury caused atherosclerotic occlusion and significant decreases in iliac artery and bladder blood flow in the 8, 12 and 16 weeks CBI groups compared with the age-matched controls. Bladder vascular density increased in the early stage (8 weeks) and mid-term (12 weeks) bladder ischemia groups but significantly diminished after prolonged ischemia (16 weeks). Masson's trichrome staining showed no structural change after 8 and 12 weeks of ischemia but significant increase in collagen deposition after 16 weeks of ischemia. Immunohistochemical staining showed an increase in VEGF and decrease in TGF-beta expression in early stage ischemia and a decrease in VEGF and increase in TGF-beta expression after prolonged ischemia. VEGF appeared to dominate in the urothelium while TGF-beta was more prominent in the suburothelial tissue. EIA showed that bladder VEGF content significantly increased in early stage ischemia and significantly decreased in the later stages of ischemia. RT- PCR analysis showed that VEGF gene expression significantly increased in early stage ischemia but significantly decreased after prolonged ischemia. VEGFR1 gene expression was unchanged throughout the course of bladder ischemia. VEGFR2 gene expression, however, increased at week 8 and then gradually decreased at 12 and 16 weeks after ischemia.

**Conclusions**

Similar changes in VEGF and VEGFR2 with varying duration of ischemia would suggest VEGF regulation of VEGFR2 expression. The bladder reacted to ischemia with an initial increase in VEGF and VEGFR2, a decrease in TGF-beta and an increase in vascular density, possibly, to preserve its blood supply and protect itself from ischemic damage. This local defensive mechanism, however, failed to restore bladder blood flow to normal, which may be due to occlusive disease of major bladder arteries. Prolonged exposure to ischemia downregulated bladder VEGF and VEGFR2 while increasing TGF-beta expression. These growth factor changes in prolonged bladder ischemia appeared to result in vascular degeneration and collagen accumulation, which may lead to non-compliance of the bladder wall.