

PROINFLAMMATORY CYTOKINES UPREGULATE CONNEXIN43 EXPRESSION AND PROMOTE GAP JUNCTION INTERCELLULAR COMMUNICATION IN BLADDER SMOOTH MUSCLE CELLS

Hypothesis / aims of study

Acute cystitis is one of the common diseases in clinic, which produces urodynamic symptoms similar to overactive bladder (OAB). The molecular mechanisms for the symptoms are presently unclear. Bladder smooth muscle cells (BSMCs) are coupled by gap junctions (GJs) and altered GJs contribute to voiding symptoms of OAB. Given that inflammatory stimulation is an important factor involved in the regulation of gap junction. We examined the effects and involved signaling pathway of proinflammatory cytokines on the expression and function of connexin 43 (Cx43) in vitro and in vivo.

Study design, materials and methods

BSMCs were treated with lipopolysaccharide (LPS), interleukin-1 beta or tumor necrosis factor alpha. Agents that interfere with NF-kappa B, cAMP signaling pathway, iNOS and COX-2 were used for intervention. Intraperitoneal injection of *E. coli* lipopolysaccharide (LPS) into mouse was used to induce bladder infection. Cx43 expression was examined by Western blot, Northern blot and immunohistochemistry. Scrape-loading Dye transfer was used to evaluate gap junctional intercellular communication (GJIC).

Results

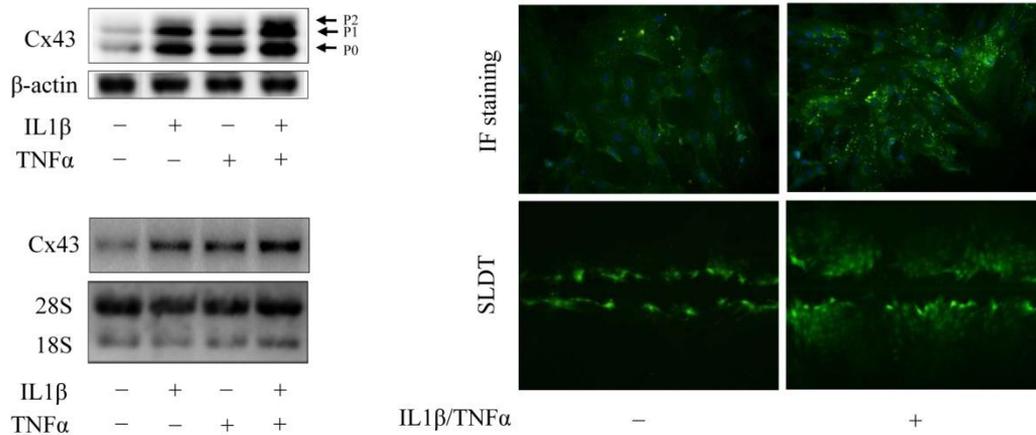
1) Incubation of BSMCs with IL-1 beta or TNF alpha increased connexin43 (Cx43). These effects of the cytokines were synergistic when in combination. 2) IL-1 beta /TNF alpha activated PKA, as revealed by VASP phosphorylation. Blockade of cAMP pathway with PKA inhibitor H89, cAMP antagonist Rp-cAMP or downregulation of cAMP response element binding protein (CREB) with siRNA largely abolished the effects of the cytokines. 3) IL1 beta /TNF alpha induced an NF-kappa B-dependent release of Cx43-stimulating activity. Inhibition of NF-kappa B-regulated gene products iNOS and COX-2 significantly abrogated, whereas exogenous NO donor SNAP and prostacyclin (PGI₂) mimicked the effects of these cytokines. 4) Intraperitoneal injection of LPS into mouse induced bladder Cx43 expression, which was significantly reduced by iNOS inhibitor L-NAME.

Interpretation of results

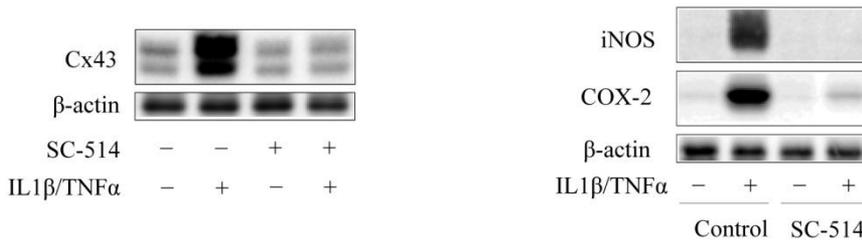
Our study demonstrated that IL-1 beta/TNF alpha induced Cx43 expression and function in BSMCs both in vivo and in vitro. This effect was mediated by cAMP signalling pathway. The complete abolishment of the Cx43-elevating effect by NF-kappa B inhibitor indicated an involvement of NF-kappa B-regulated gene products. The possible candidates could be NO and PGI₂, which were shown by the observations that: 1) iNOS and COX-2 induced by inflammatory cytokines; 2) iNOS and COX-2 inhibitors partially blocked the effects of the cytokines; and 3) NO donor and PGI₂ also induced Cx43 expression.

Concluding message

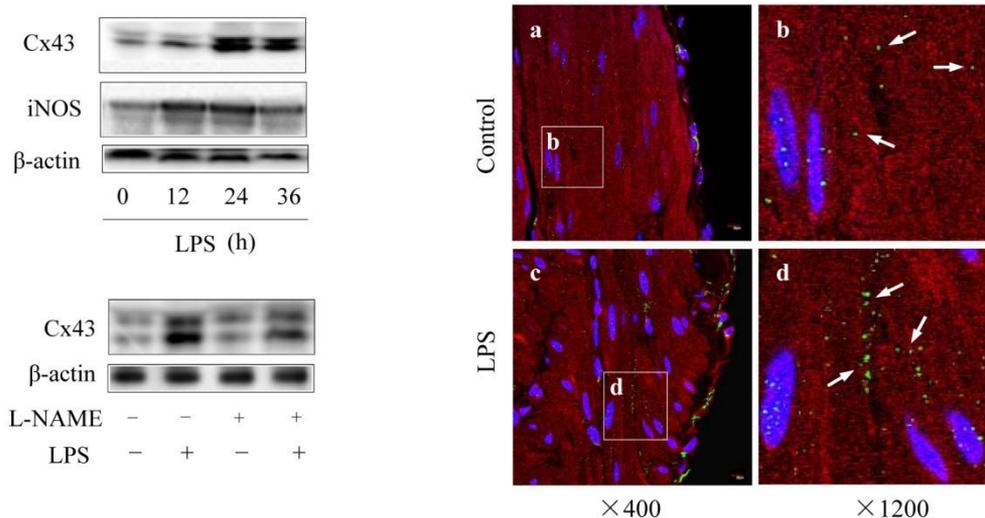
We demonstrate that inflammatory stimulation potently induced Cx43 in BSMCs through NFkB/cAMP/PKA signaling cascade. Our findings thus provide a potentially important molecular mechanism for urinary frequency and urgency of bladder infection. In addition, it may open a new window on our understanding of inflammatory urinary disorders.



Effects of proinflammatory cytokines on Cx43 expression and gap junctional intercellular communication (GJIC).



Induction of a nuclear factor- κ B (NF κ B)-dependent release of Cx43-elevating activity by IL1 β /TNF α . (SC514- NF-kappa B inhibitor)



Induction of bladder Cx43 expression by intraperitoneal injection of LPS into mice

Specify source of funding or grant	None
Is this a clinical trial?	No
What were the subjects in the study?	ANIMAL
Were guidelines for care and use of laboratory animals followed or ethical committee approval obtained?	Yes
Name of ethics committee	All animal procedures were reviewed and approved by the University of Yamanashi Animal Care and Use Committee. All efforts were made to minimize animal suffering and to reduce the

number of animals used.
