

## **ACTIVATION OF TOLL LIKE RECEPTOR 7 INDUCES CYSTITIS AND FACILITATION OF BLADDER PAIN AND MECHANO-SENSATION IN MICE**

### Hypothesis / aims of study

Toll like receptors (TLRs) function as sentinels, alerting the innate immune system in the presence of threatening microbial invasion. Among them, TLR7 is mainly expressed in neural and inflammatory cells, but also in the human and mouse bladder urothelium, and responsible for the sensation of pain and induction of inflammation (1, 2). It has been proposed that TLR7 is involved in some auto-immune diseases such as systemic lupus erythematosus and Sjögren's syndrome (3), well-known accompanied diseases with interstitial cystitis. These previous findings make us presume that TLR7 may have a pathophysiological role in the development of bladder pain and interstitial cystitis. However, to date, there has been no report about the role of TLR7 in the bladder sensory and inflammatory disorders. Therefore, we investigated the effects of TLR7-activation provoked by intravesical instillation of its selective agonist, loxoribine (LX), on nociception, mechano-sensation and histology of the bladder in mice.

### Study design, materials and methods

Female C57/BL6N mice (mean body weight: 19.6 ± 0.2 g) were used. In the assessment of bladder pain-like behaviour, the numbers of licking close to the bladder were counted for a 30-minutes period before and at several time-points after intravesical instillation with LX (1.5 or 4.5 mM) or its vehicle. In the frequency volume (FV) measurements, the mice were placed individually at a metabolic cage and monitored their voiding behaviour for 24 hours as a base-line. Then, the mice were received intravesical instillation of LX (1.5 or 4.5 mM), and placed back to the cage and voiding behaviour was monitored further 3 days. In the cystometry (CMG) measurements, the mice underwent the supracolicular decerebration under anesthesia with isoflurane. After the recovery from the anesthesia (> 2 hours), continuous CMG was performed with saline-instillation as a base-line. Then, measurements were repeated with LX-instillation (1.5 mM). In the afferent measurements, mechanosensitive multiple pelvic nerve activities from the isolated bladder were analyzed by Spike2 program to classify the single-unit response to bladder distension. After the base-line measurement of the nerve activity with saline-instillation, the measurements were repeated 3 times with LX-instillation (1.5 mM). At the end of the CMG and FV measurements, the bladder was harvested and histological evaluations were performed.

### Results

LX-instillation increased the number of licking behaviour compared with the vehicle-instillation, which had two-peaks; in the acute (2 hours) and subacute (day 2-3) phases (Figure 1A). In the FV measurements, after LX-instillation, voiding frequency increased gradually in a dose-dependent manner and mean voided volume gradually decreased at 4.5 mM (Figures 1B and 1C). In the CMG measurements, LX-instillation reduced inter-contraction interval and voided volume (Table 1 and Figure 2A). In the afferent measurements, LX-instillation facilitated the afferent activities in response to bladder distention (Figure 2B). Histological examinations demonstrated that inflammatory cell infiltration, edema and hemorrhage in the suburothelial layer were observed in the subacute phase (day 3), whereas there were no remarkable changes in the acute phase (2 hours) (Figure 3).

### Interpretation of results

Intravesical LX-instillation immediately induced frequent licking as a pain-like behaviour, and urinary frequency in the CMG measurements and facilitated the mechanosensitive afferent activities, suggesting that activation of bladder TLR7 acutely facilitates nociceptive and mechano-sensitive afferent pathways innervating the bladder. Moreover, intravesical LX-instillation also induced inflammatory changes in the suburothelial layer of the bladder as a delayed response, which was accompanied with increased voiding frequency and reduced voided volume and increased nociceptive behaviour at 2-3 days after instillation, implying that bladder TLR7-activation also induces bladder inflammation leading to facilitation of bladder pain and mechano-sensation in a subacute phase.

### Concluding message

The present results indicate that activation of the bladder TLR7 can induce cystitis and facilitation of the bladder mechano-sensory and nociception pathways in mice.

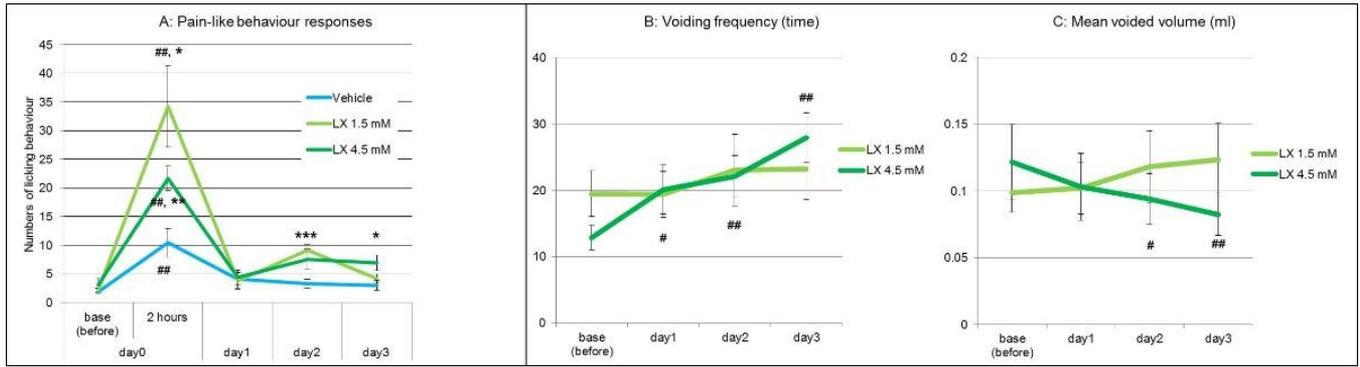


Figure 1. The numbers of licking behaviour before and after LX (1.5 mM or 4.5 mM) and its vehicle (water) instillation in the mice bladder (N = 6 in each group) (A). Changes of voiding behaviour after intravesical instillation of LX at 1.5 mM (N = 8) or 4.5 mM (N = 9) in the FV measurements (B and C)

#P <0.05, ##P <0.01: significant differences from base (repeated measures ANOVA followed by Dunnett's test)

\*P <0.05, \*\*P <0.01, \*\*\*P <0.001: significant difference vs. vehicle (unpaired Student's t-test)

Table 1. The effects of intravesical LX-instillation on CMG parameters

	BP (cmH <sub>2</sub> O)	TP (cmH <sub>2</sub> O)	MP (cmH <sub>2</sub> O)	ICI (second)	VV (ml)
Base-line	2.4 ± 0.3	9.1 ± 0.6	29.7 ± 1.9	681 ± 78	0.11 ± 0.01
LX 1.5 mM	2.7 ± 0.4	7.0 ± 0.5 <sup>†</sup>	27.3 ± 1.9	405 ± 30 <sup>‡</sup>	0.07 ± 0.01 <sup>‡</sup>

The values are expressed as mean ± S.E.M. (N = 7).

BP: basal pressure, TP: threshold pressure, MP: maximum pressure, ICI: intercontraction interval, VV: voided volume,

<sup>†</sup>P <0.05, <sup>‡</sup>P <0.01: significant differences from base (paired Student's t-test)

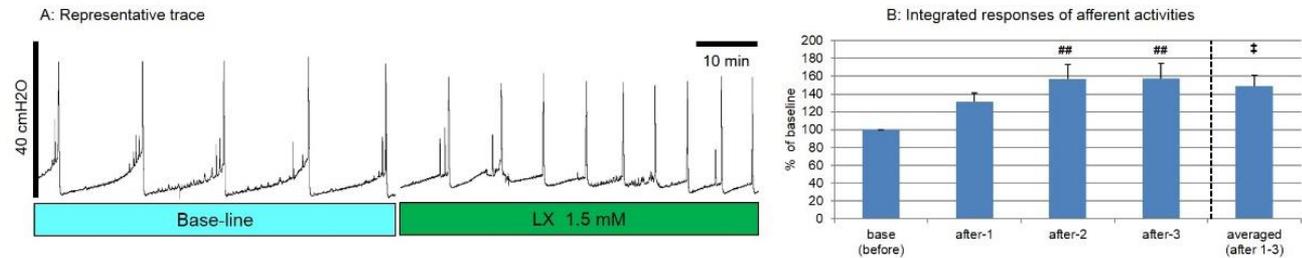


Figure 2. Representative CMG recordings with intravesical instillation of LX at 1.5 mM in a decerebrated unanesthetized mouse (A) and mechanosensitive afferent activities of the pelvic nerve in integrated responses (N = 6, n = 31) (B)

##P <0.01: significant difference from base (repeated measures ANOVA followed by Dunnett's test), <sup>†</sup>P <0.05: significant difference from base (paired Student's t-test)

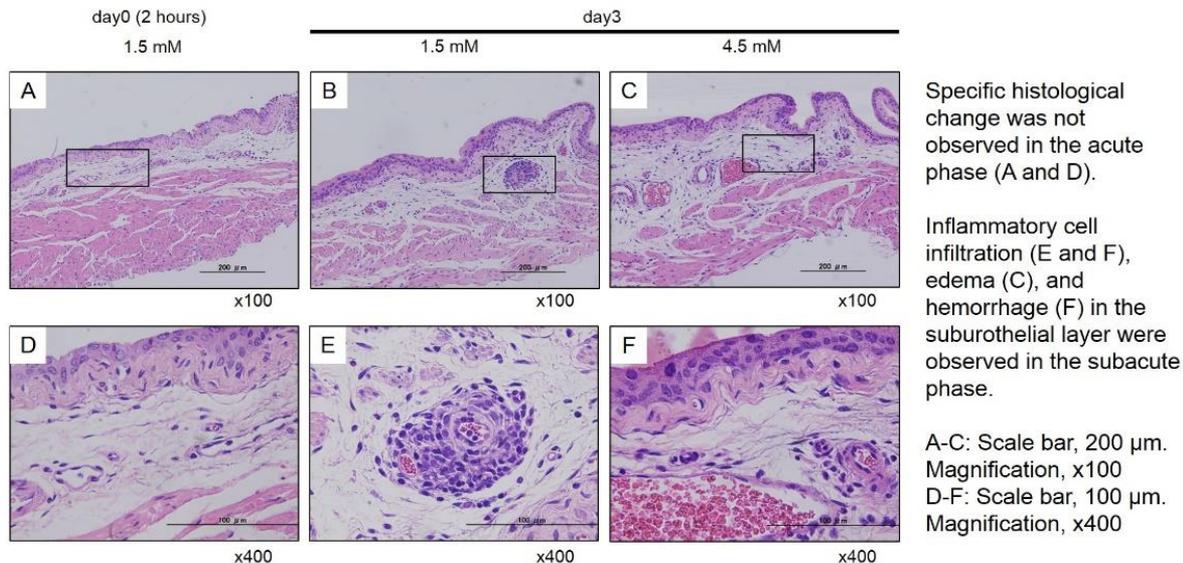


Figure 3. Representative histological findings after intravesical instillation of LX at 1.5 mM (A and D) in the acute phase (2 hours) and LX at 1.5 mM (B and E) or 4.5 mM (C and F) in the subacute phase (day 3)

## References

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Disclosures

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