352

Funahashi Y[1], Yoshida M[2], Yamamoto T[1], Matsukawa Y[1], Takai S[1], Gotoh M[1]
1. *Nagoya University,* ***2.*** *National Center for Geriatrics and Gerontology*

RECOVERY OF UROTHELIAL BARRIER FUNCTION BY REBAMIPIDE

Hypothesis / aims of study

Rebamipide, (2-[4-chlorobenzylamino]-3-[2{1H}-quinolinon-4-yl] propionic acid, Otsuka Pharmaceutical Co., Tokyo, Japan) is used orally for treatment of gastritis, as eye drops for dry eye, and as an enema for inflammatory bowel diseases. Its mechanism of action includes inhibiting inflammation, accelerating wound healing, and protecting the mucosa. For these pharmacological effects, high local concentration has to be achieved in the target tissue. We subsequently hypothesized that topical treatment with rebamipide might be effective for urothelial repair. In this study, we examined the healing effects of intravesical application of rebamipide on a damaged urothelium using a chemically induced cystitis rat model.

Study design, materials and methods

Female SD rats were injected into the bladder with hydrochloride to induce cystitis. On days 1 and 4, they were administered with rebamipide (1 or 10 mM) or vehicle into the bladder, and then kept for 1 h. Histopathology, urothelial permeability, cystometrogram, and nociceptive behaviors were evaluated on day 7. In addition, the tissue rebamipide concentrations after the 1 h bladder instillation were quantified using high-performance liquid chromatography.

Results

Intravesically administered rebamipide permeated the bladder particularly in hydrochloride-treated rats, and the pharmacologically effective tissue dose remained for >6 h. Bladder histological evaluation revealed that polymorphological inflammatory cells infiltrated the submucosal layer of hydrochloride-treated rats (Fig. 1). Immunohistochemistry showed decreased positivity for uroplakin 3A along the inner layer of the urothelium by hydrochloride treatment (Fig. 2). Scanning electron microscopy revealed that tight junctions were damaged in the hydrochloride-treated rats (Fig. 3). The absorption of Evans Blue into the bladder wall was increased in hydrochloride-treated rats (Fig. 4). These findings, which were associated with urothelial injury and increased permeability, were suppressed by rebamipide treatment dose-dependently. Cystometrograms demonstrated that the intercontraction interval was shorter in hydrochloride-treated rats, but was prolonged by rebamipide (Fig. 5). The increased nociceptive behaviors observed after intravesical resiniferatoxin administration were also suppressed by rebamipide (Fig. 6).

Interpretation of results

This study revealed five major findings. First, rebamipide administered inside the bladder directly permeated the bladder wall. This effect was more pronounced in the cystitis model than normal rats. Importantly, a pharmacologically effective concentration of rebamipide persisted for >6 h in the cystitis model. Second, intravesical application of rebamipide accelerated the healing of damaged urothelial cells and tight junctions. Third, it enhanced the recovery of urothelial permeability, which resulted in decreased submucosal inflammation. Fourth, the protection of the urothelial barrier resulted in reduced pain reactions against bladder nociceptive stimuli. Finally, rebamipide was also effective for bladder overactivity.

The hydrochloride-induced cystitis rat model is used as an interstitial cystitis/painful bladder syndrome (IC/PBS) model because they share similar features. Although the pathogenesis of IC/PBS is not fully understood, increased urothelial permeability is a key factor. The damaged urothelium allows the transepithelial migration of solutes such as potassium, which stimulates subepithelial afferent nerves and causes irritative symptoms. The leaking of toxic solutes through the defective urothelium can activate pain-sensing C-fibers located within the urothelium and submucosa of the bladder. These findings suggest that protecting urothelial barrier can be a therapeutic target.

Current systemic therapies for IC/PBS include physical therapy, oral tricyclic antidepressants, anti-histamines, pentosan polysulfate sodium, and non-steroidal anti-inflammatory drugs. The effects of these therapies are usually modest; therefore, several local treatments including the intravesical application of dimethyl sulfoxide, resiniferatoxin, and heparin, or the intra-detrusor injection of botulinum toxin in addition to bladder hydrodistention are performed clinically. The intravesical application of rebamipide is not highly invasive and could be used repeatedly; thus, we assume it could become a useful treatment for IC/PBS if approved clinically.

Concluding message

Intravesical administration of rebamipide relieved bladder overactivity and nociception in a hydrochloride-induced cystitis model, which was accompanied by accelerated urothelial repair. This could be a novel strategy for the treatment of patients with IC/PBS.

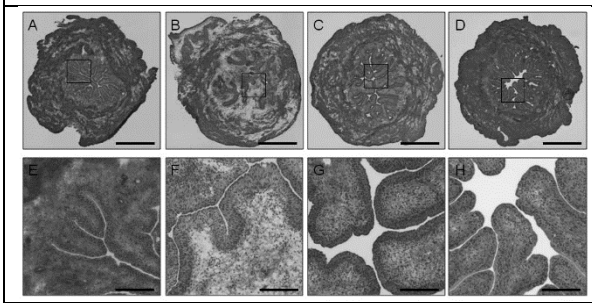
Fig. 1 H&E staining of the bladder	
	Compared with control (A and E), increased submucosal edema and inflammatory cell infiltrates were observed in hydrochloride-treated rats (B and F). This inflammation was ameliorated by the intravesical administration of 1 mM (C and G) and 10 mM rebamipide (D and H). Scale bar: 1 mm (A–D) and 200 μm (E–H).

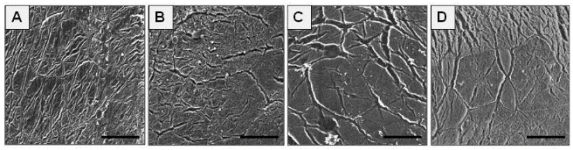
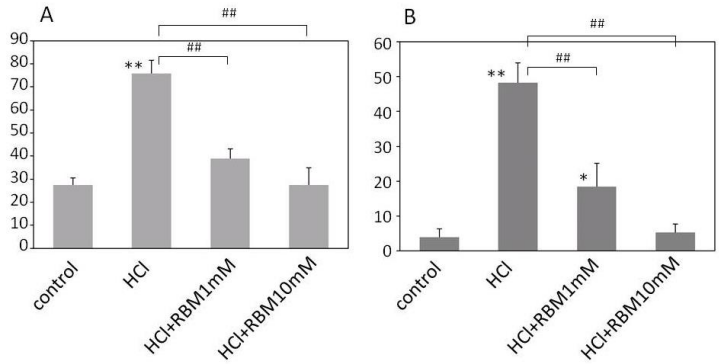
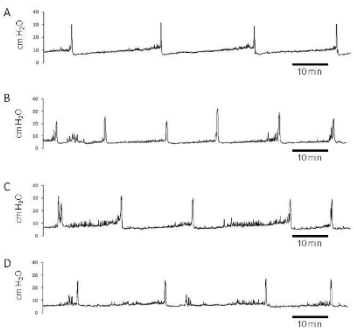
Fig. 2 Scanning electron microscopy findings	
	Cell borders on the urothelial surface of control rats (A) were disappered with smooth cell surface in hydrochloride-treated rats (B). These changes to the surface of the bladder were reduced in rats treated with 1 (C) and 10 mM (D) rebamipide. Scale bar: 20 μm.

Fig. 3 Nociceptive behaviors	*Fig. 4 Cystometry*
	
Nociceptive behavioral studies were performed to evaluate licking (A) and freezing behaviors (B). The incidence of both licking and freezing behaviors was increased in HCl-treated rats. Nociceptive behaviors decreased in rebamipide-treated rats. # $p < 0.05$ between groups.	The intercontraction interval was significantly shorter in HCl group (B) compared to control (A). Rebamipide prolonged micturition interval. C; 1 mM, D; 10mM

Disclosures
Funding: None **Clinical Trial:** No **Subjects:** ANIMAL **Species:** Rat **Ethics Committee:** Nagoya University Institutional Animal Care and Use Committee