Intravesical Application of Rebamipide Suppresses Bladder Inflammation in a Rat Cystitis Model

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Purpose: We examined the effects of intravesical application of rebamipide (Otsuka Pharmaceutical, Tokyo, Japan) on bladder inflammation and overactivity in a chemically induced cystitis model.

Materials and Methods: Female Sprague Dawley® rats under isoflurane anesthesia were injected with 150 mg/kg cyclophosphamide in the peritoneum, and 1 mM or 10 mM rebamipide or vehicle was administered in the bladder and remained for 1 hour. Control rats were injected with saline in the peritoneum and vehicle was administered in the bladder. The bladder was harvested at 48 hours. Hematoxylin and eosin staining was performed and the inflammation grade was assessed. The amount of myeloperoxidase was measured using enzyme-linked immunosorbent assay. Proinflammatory cytokines were quantified using reverse transcriptase-polymerase chain reaction. Cystometrogram was done in awake rats 48 hours after cyclophosphamide treatment to measure voiding reflex parameters.

Results: Histological evaluation revealed that bladder inflammation in cyclophosphamide treated rats was suppressed by rebamipide in a dose dependent manner. Up-regulated myeloperoxidase, IL-1β, IL-6 and TNF-α expression in cyclophosphamide treated rats was also suppressed in rebamipide treated rats. Cystometrogram demonstrated that the intercontraction interval decreased in cyclophosphamide treated rats but was prolonged by rebamipide.

Conclusions: Intravesical application of rebamipide suppressed bladder inflammation and overactivity in a dose dependent manner. This may provide a new treatment strategy for chemotherapy associated cystitis.

Key Words: urinary bladder, overactive; inflammation; cystitis; rebamipide; cyclophosphamide

Abbreviations and Acronyms

CYP = cyclophosphamide

GAPDH = glyceraldehyde-3-phosphate dehydrogenase

ICI = intercontraction interval

IL = interleukin

MPO = myeloperoxidase

TNF = tumor necrosis factor

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Study received Nagoya University institutional animal care and use committee approval.

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REBAMIPIDE has been widely used for acute and chronic gastritis and ulcer disease. Its mechanism includes suppression of inflammation, proinflammatory cytokines and chemokines, inhibition of inflammatory cell activation and migration, inhibition of free radicals and reactive oxygen species, acceleration of wound healing and mucosal barrier repair, and stimulation of prostaglandin and mucus glycoprotein synthesis.[1–3]

Because these bioregulation effects of rebamipide, including cytoprotective, wound healing and anti-inflammatory properties, may be common in various organs, rebamipide may have protective and healing actions in various tissues. Most orally administered rebamipide is not

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absorbed from the intestine[4] and rebamipide acts on damaged organs by direct contact. Thus, it must be administered locally to exert its effects. Several animal studies demonstrated its anti-inflammatory effects in the stomach as well as the colon,[5] intestines,[6] lungs[7] and conjunctiva[8] in animal models when administered directly to these target organs. Rebamipide was also recently approved for dry eyes in the form of eye drops and groups reported the potency of a rebamipide enema against inflammatory bowel disease.[9–11]

Based on this background we hypothesized that rebamipide may effectively suppress bladder inflammation when given intravesically. Specifically, we applied rebamipide directly in the bladder in a chemically induced cystitis rat model and investigated its therapeutic effects on bladder inflammation and overactivity.

MATERIALS AND METHODS

All animal experiments were performed in accordance with institutional guidelines and approved by the Nagoya University Institutional Animal Care and Use Committee.

Cystitis Model

Female Sprague Dawley rats weighing 230 to 260 gm were injected with CYP (150 mg/kg) in the peritoneum while under isoflurane anesthesia. The bladder was compressed and emptied. Rebamipide (300 μl, 1 mM or 10 mM) or vehicle composed of 1.000 gm polyvinyl alcohol, 0.146 gm sodium citrate, 0.715 gm sodium chloride and 0.180 gm potassium chloride in distilled water (adjusted to pH 6, total amount 100 ml) was administered in the bladder. Rats were kept supine for 1 hour under anesthesia. Control rats were injected with saline in the peritoneum and vehicle was administered in the bladder. The rats were divided into 4 groups, including control, CYP, CYP plus 1 mM rebamipide and CYP plus 10 mM rebamipide.

Animal Perfusion and Tissue Preparation

At 48 hours after intraperitoneal CYP administration 24 rats (6 per group) were anesthetized with isoflurane, followed by intracardiac perfusion with cold heparinized saline. The bladder was excised immediately.

Histological Analysis

The excised bladder was immediately frozen in frozen section compound (Leica Microsystems, Wetzlar, Germany), cut into 8 μm sections and mounted on slides. Tissue sections were fixed in 4% paraformaldehyde for 10 minutes, stained with hematoxylin and eosin, dehydrated through a graded ethanol series, cleared in xylene and coverslipped with mounting medium (Merck, Darmstadt, Germany). Bladder histological changes were graded as 0—no evidence of inflammatory infiltration or interstitial edema, 1—mild (few inflammatory cells and little or no interstitial edema), 2—moderate (infiltration of a moderate number of inflammatory cells and moderate interstitial edema) or 3—severe (many diffuse infiltrating inflammatory cells and severe interstitial edema). A single blinded research technician at our laboratory graded bladder histology.

MPO Assay

After histological evaluation the bladder was kept at −80C until mRNA or protein analysis was done. Half of the bladder tissues were homogenized in RIPA lysis buffer. The homogenate was centrifuged at 10,000 × gravity for 10 minutes and supernatants were stored at −80C until assayed. MPO concentration was measured using an enzyme-linked immunosorbent assay kit (Hycult® Biotech). Protein concentration was determined using a Bio-Rad® kit with bovine serum albumin as the standard. MPO concentrations were standardized to tissue protein levels and expressed as ng/mg total protein.

Cytokine mRNA Quantification

Total RNA was extracted from the other half of the bladder tissue using TRIzol® reagent according to the manufacturer protocol. RNA concentration was estimated by measuring absorbance at 260 nm with a NanoDrop™ 2000c. The 260/280 nm absorbance ratio was used to check for purity. RNA (1 μg) was reverse transcribed into cDNA using SuperScript™ II. Primer and probe sets designed for TaqMan® Gene Expression Assays were used for IL-1β, IL-6, TNF-α and GAPDH. mRNA levels were quantified with a Mx3000P™ Real-Time PCR System in a 20 μl volume using PCR Master Mix (Applied Biosystems®). We amplified cDNA for 40 cycles at 95C for 15 seconds and 60C for 60 seconds. Reaction specificity was confirmed by melting curve analysis. Samples were quantified using the threshold cycle ratio in regard to GAPDH.

Cystometry

At 48 hours after intraperitoneal CYP administration 6 rats per group were anesthetized with isoflurane. Via a lower midline abdominal incision we exposed the bladder and inserted PE-50 tubing (Clay Adams, Parsippany, New Jersey) in the bladder through the dome. Anesthesia was ended and unrestrained conscious rats were placed in a recording cage. After 2-hour acclimation saline was infused transvesically at 0.04 ml per minute and the rats voided spontaneously through the urethra. At least 4 reproducible micturition cycles were recorded after an initial 60-minute stabilization period. Chart™ 7 was used for data collection and analysis. We evaluated baseline pressure, voiding pressure threshold, peak voiding pressure and ICI.

Statistics

All values are expressed as the mean ± SE. The nonparametric Mann-Whitney U test was used to assess differences between groups. All tests were 2-sided with $p < 0.05$ considered statistically significant. Statistical analysis was done with SPSS®.

RESULTS

Histopathology

Inflammatory changes, including severe submucosal edema and inflammatory cell infiltration accompanied by urothelial injury, were observed in the