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Bacterial Infection and Acid Mucopolysaccharides in Epithelium of Rat Urinary Bladder

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Summary. The distribution of acid mucopolysaccharides in bladder epithelium was studied by colloidal iron staining in experimental cystitis of rats. In normal bladders the staining was mainly confined to the superficial (luminal) layer, but observed uniformly in all layers of epithelium in cystitis. Enzyme digestion (hyaluronidase, heparitinase, chondroitinase AC, chondroitinase ABC) disclosed the existence of hyaluronic acid, heparan sulphate and dermatan sulphate in the bladder epithelium. The possible roles of these mucopolysaccharides glycosaminogycans) were discussed.

Key words: Acid mucopolysaccharides, Bacterial cystitis, Glycosaminoglycans, Rat urinary bladder.

Introduction

Mucopolysaccharides (glycosaminoglycans) have been demonstrated in the epithelium of the urinary bladder of the rat [6, 3], rabbit [11] and in man [8]. Histochemical findings that the epithelium is stained by PAS, alcian blue and colloidal iron indicate that the mucopolysaccharides consist of acid mucopolysaccharides (acidic glycosaminoglycans). It has been suggested that the biological role of mucopolysaccharides is to act as the primary defence mechanism to prevent bacterial attachment to the luminal epithelium, [10]. However, the relationship between these materials and bacterial infection still remains obscure.

The aim of the present experiment was to study these mucopolysaccharides in the bladder epithelium following bacterial infection and to identify the kind of acid mucopolysaccharides.

Materials and Methods

Preparation of Bacteria

E. coli type 07 isolated from a patient's urine at Teishin Hospital, Sapporo, Japan, was incubated in brain-heart infusion broth (E-MC07, Eiken, Japan), at 37 °C for 24 h. The washing procedure was repeated and a bacterial suspension of 10^9 E. coli per ml of broth was prepared.

Animals

29 male Wistar rats, weighing 230 to 270 g, were used. They were divided into the following three groups: Group 1, eight rats for control sham operation; Group 2, eight rats for placement of a foreign body, a sterile polyethylene tube (1 mm of length, 1 mm of diameter), in the bladder; Group 3, eight rats for bacterial innoculation in the bladder with a foreign body.

Surgical Procedures

The rats were anaesthetised with pentobarbital (50 mg/kg, I.M.). Through a mid-line abdominal skin incision the bladder was exposed. All urine in the bladder was aspirated through a 27 gauge hypodermic needle and transfered to a culture medium (brain-heart infusion agar, E-MC06, Eiken, Japan) for bacterial examination. In a case of positive bacterial examination, the animal was omitted from the experimental group. In the control sham operation, the bladder was opened for a distance of 2 mm with small scissors, then simply closed with a one layer continuous suture of 0-6 Dexon. For the bacterial inoculation with a foreign body, 0.1 ml of the prepared bacterial suspension broth was injected into the bladder through a 27 gauge hypodermic needle after the placement of the foreign body and the closure of the bladder.

All procedures were performed under aseptic conditions. Rats were fed animal diet (MF-food, Oriental Yeast Co., Japan) and tap water freely. Rats in each group were stunned to death at each of the following postoperative periods; 3 h, 6 h, 12 h and 2 weeks. The bladder urine was again transfered to the culture medium for bacterial examination.

Staining procedures

The tissue was fixed in Bouin's solution. The deparaffinised section was stained with HE and colloidal iron [7].

Enzyme digestion

Hyaluronidase (Sigma, U.S.A.), heparitinase (extracted and purified by affinity chromatography at the Department of Biochemistry,

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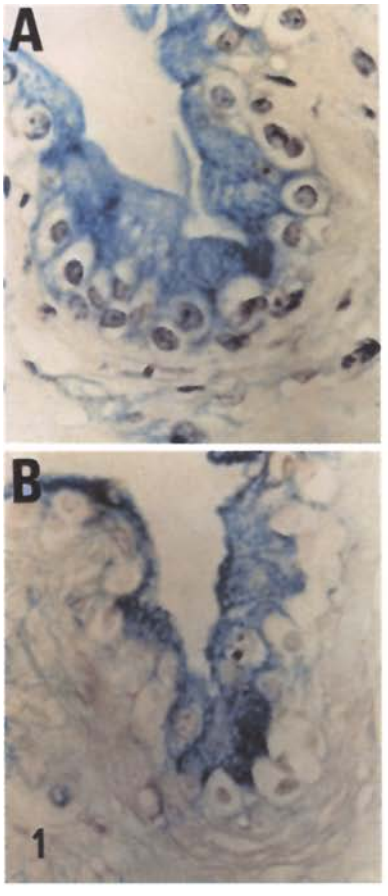

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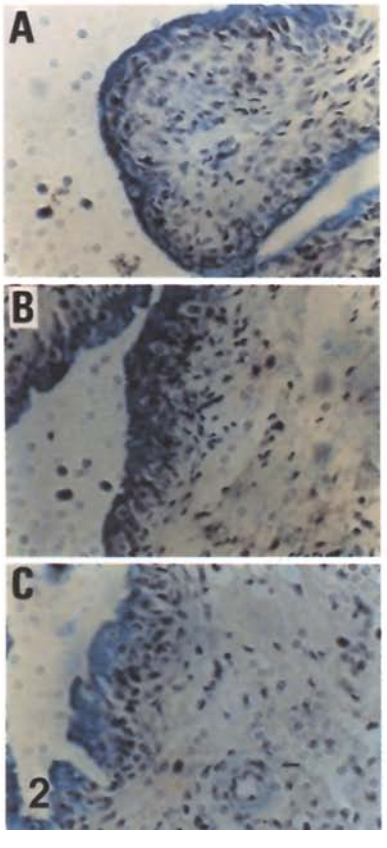

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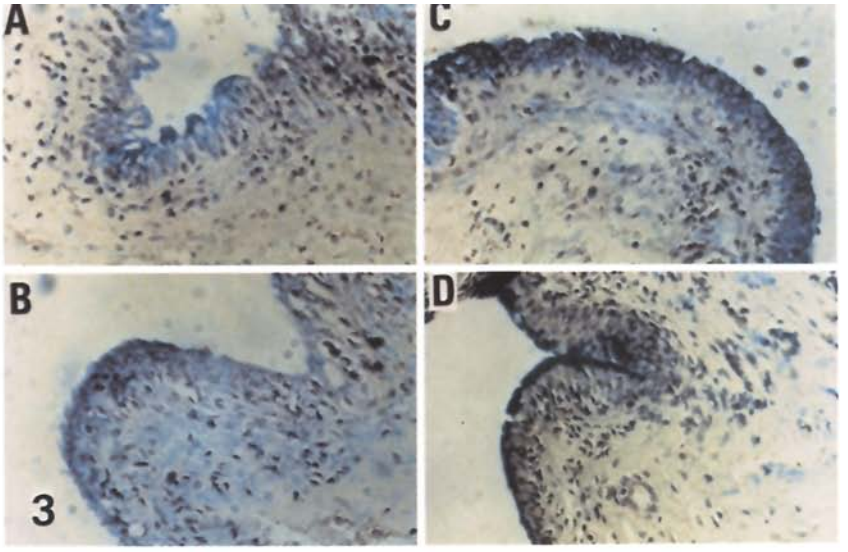

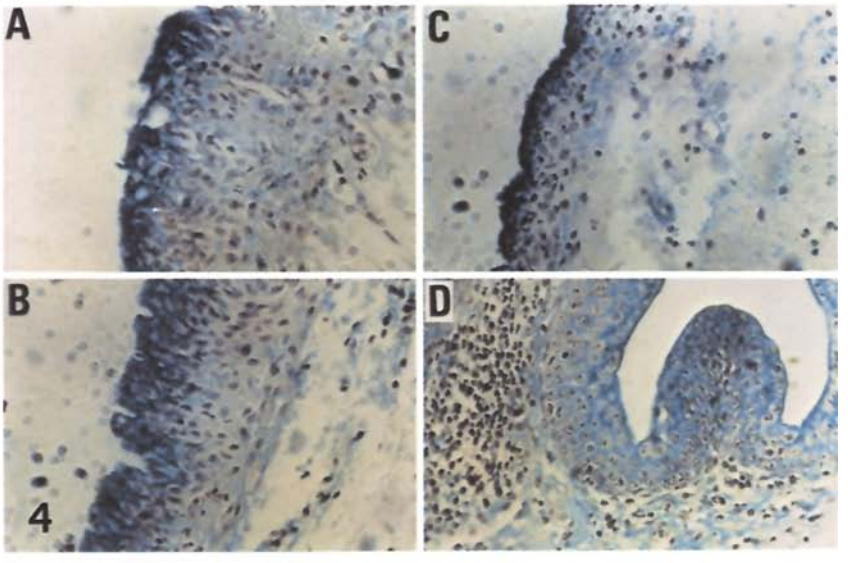

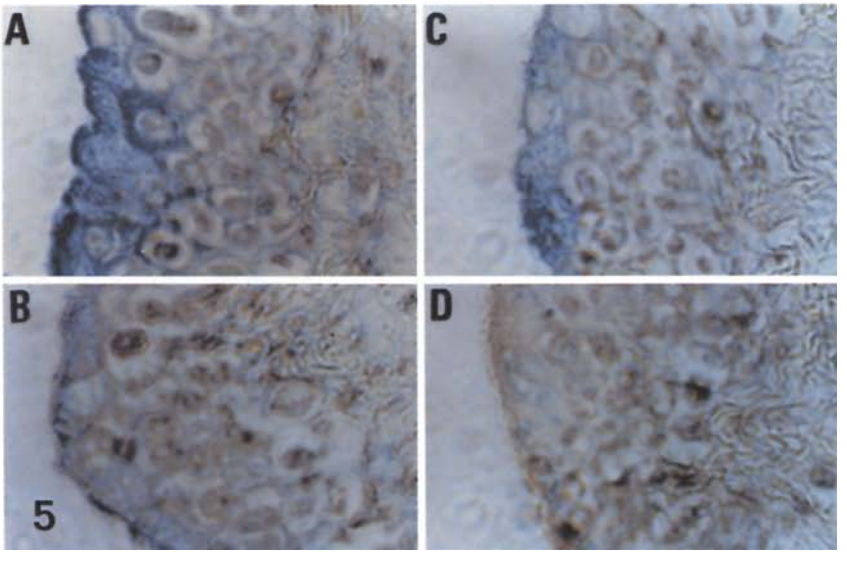

Tohoku University School of Medicine [9]), chondroitinase AC and chondroitinase ABC (Seikagaku Fine Biochemicals, Japan) were used for enzyme digestion. For hyaluronidase digestion, deparaffinised sections treated with the enzyme in 0.1 M acetic buffer (pH 5.0) overnight at 37 °C. For heparitinase treatment, sections were treated in the same manner in 0.1 M sodium acetate buffer (pH 7.0). For chondroitinase treatment sections were treated in the same manner in 0.1 M tris buffer (pH 7.2). Control sections were treated only with the buffer solution.

Results

1. Colloidal Iron Staining in the Bladder Epithelium

Normal Controls. The cytoplasm of the bladder epithelium showed a positive reaction with colloidal iron, especially the superfical (luminal) layer as shown in Fig. 1A. After treatment with hyaluronidase the colloidal iron positive substances in the deeper layer were slightly digested and the laterality of the reaction in the superficial layer was more distinct compared to that in the deeper layer as shown in Fig. 1B.

Sham Operation. The colloidal iron-positive cellular arrangement was not markedly changed for 3 h, 6 h and 12 h compared to the normal control as shown in Fig. 2. Urine recovered from each postoperative periods was sterile.

◀ **Fig. 1A, B.** Distribution of colloidal iron in normal rat bladder. Colloidal iron is demonstrated as a bright blue staining. It is mainly confined to the superficial (luminal) layer of epithelium (**A**). The laterality of its distribution becomes distinct after hyaluronidase digestion (**B**). (Original magnification x 360)

Fig. 2 A–C. Distribution of colloidal iron in sham operation. Three h (**A**), 6 h (**B**) and 12 h (**C**) of postoperative period. The distribution is not changed compared to normal control. (Original magnification x 180)

Fig. 3A–C. Distribution of colloidal iron in placement of foreign body. Three h (**A**), 6 h (**B**), 12 h (**C**) and 2 weeks (**D**) of postoperative period. The distribution is not changed in (**A**) compared to normal control. In (**B**) and (**C**) the colloidal iron is observed in submucosa. In (**D**) the distribution is again confined, even more prominent, in superficial (luminal) layer. (Original magnification x 180)

Fig. 4A–C. Distribution of colloidal iron in bacterial inoculation with foreign body. Three h (**A**), 6 h (**B**), 12 h (**C**) and 2 weeks of postoperative period. The distribution of colloidal iron is not markedly changed in (**A**) and (**B**). In (**C**) the colloidal iron is observed in deeper layer of epithelium. Leucocytes are observed sparsely in the epithelium and submucosa. In (**D**) the colloidal iron is observed uniformly in all the layer of epithelium and leucocytes are massive in the submucosa. (Original magnification x 180)

Fig. 5A–C. Enzyme digestion. Control treated solely by buffers is shown in (**A**). Colloidal iron positive substances are partly digested by heparitinase (**B**). Further digestion by chondroitinase AC shows no effect (**C**). Further digestion by chondroitinase ABC makes the colloidal iron positive substances disappear (**D**). Details see text. (Original magnification x 360)

Placement of a Foreign Body. The colloidal iron-positive cellular arrangement remained almost the same as after the sham operation. However, the colloidal iron positive staining could be observed also in the submucosal layer at 6 h (Fig. 3B) and 12 h (Fig. 3C). For 2 weeks the distribution of colloidal iron positive staining was again confined to the superficial layer. At this time the positive reaction in the superficial layer was more prominent compared to the normal control (Fig. 3D). Bladder calculi deposited arround the foreign body were consistently observed. Urine recovered from each postoperative period was sterile.

Bacterial Inoculation with a Foreign Body. The colloidal iron-positive cellular arrangement remained almost the same as in the placement of a foreign body alone for the first 6 h (Fig. 4A, B). After 12 h the colloidal iron positive staining could be observed even in the deeper layer and sparse leucocyte infiltration developed in the epithelium and in the submucosa (Fig. 4C). Marked differences were seen at 2 weeks. Colloidal iron-positive staining was observed uniformly in all layers of epithelium and marked leucocyte infiltration occured in the submucosa. The connective tissue was also stained like a meshwork (Fig. 4D). Urine recovered form each postoperative period was always positive for E. coli 07.

2. Enzyme Digestion

Results of enzyme digestion are shown in Fig. 5. A control preparation treated solely by buffers is shown in Fig. 5A. The treatment with heparitinase decreased the colloidal iron staining of epithelium as shown in Fig. 5B. Treatment with chontroitinas AC had no effect on the staining as shown in Fig. 5C. However, further treatment with chondroitinase ABC made the colloidal iron staining negative as shown in Fig. 5D. These results indicate that the colloidal iron-positive substances consisted mainly of heparan sulphate and dermatan sulphate.

Discussion

By colloidal iron methods sites of acid mucopolysaccharide (gluycosaminoglycans) distribution are demonstrated as a bright blue staining. These methods are not specific for acid mucopolysaccharides, since other substances such as egg albumin, casein and peptone also show positive reaction. However, it is generally accepted that a good correlation exists between the positive reaction of colloidal iron and sites of acid mucopolysaccharide distribution, the presence of which is confirmed by other reliable procedures [13]. On the basis of these findings the colloidal iron methods are widely used for acid mucopolysaccharides.

The present findings that hyaluronidase, chondroitinase ABC and heparitinase, but not chondroitinase AC, digested the colloidal iron-positive substances in the bladder epithelium indicate the presence of hyaluronic acid, dermatan

sulphate and heparan sulphate at the site. The differences in digestion suggest that the main acid mucopolysaccharides in the superficial layer are heparan sulphate and dermatan sulphate. A study of mucopolysaccharides in the rat kidney [2] showed that heparan sulfate and dermatan sulfate accounted for most of the cortical acid mucopolysaccharides while chondroitin sulphate A (chondroitin 4-sulphate) and chondroitin C (chondroitin 6-sulphate) were not detected. These findings accord well with the results of the study in the rat bladder. Heparan sulphate is probably the same substance reported by Allalouf et al. [1] as an anticoagulant acid mucopolysaccharides in rat kidney. This substance is likely to show heparin-like property. Hanno et al. [5, 4] reported that the surface mucopolysaccharides layer of the urinary bladder interfered with bacterial attachment and that alteration of this layer by hydrochloric acid permited bacterial adsorption to the mucosa. They reported further that heparin blocked the attachment of inoculated bacteria to these acid treated bladder. In this way heparan sulphate may have a major role in interrupting bacterial attachment to normal bladder epithelium.

Acid mucopolysacchrides may also be involved in epithelial desquamation. It has been observed clinically that urine normally contains a certain number of epithelial cells desquamated from the bladder and that they are increased in cystitis [12]. This suggests that normally desquamation takes place sporadically whereas it increases in cystitis. The present findings, that colloidal iron distribution was confined to the superficial layer in normal controls found diffusely in all layers in cystitis, suggest a correlation between acid mucopolysaccharide distribution and epithelial desquamation. Epithelial desquamation may play a part in the defence mechanism of the bladder against bacterial infection. Recent electron microscopic observations of experimental cystitis reported by Fukushi et al. [3] support this possibility. They reported that superficial cells invaded by bacteria were collapsed and desquamated, and fell off into the urine.

The physiological and biological roles of mucoplysaccharides in connective tissue reported here need to be studied further.

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