# Formulation Study of Intravesical Oxybutynin Instillation Solution with Enhanced Retention in Bladder

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A formulation study of intravesical oxybutynin (OB) preparations was carried out in order to improve the effectiveness in intravesical instillation therapy for spastic neurogenetic bladder. Sodium hyaluronate (HYA) was introduced to enhance the muco-adhesiveness of the instillation preparation, and the physicochemical properties of the OB formulation were evaluated in comparison with a conventional formulation containing hydroxypropyl-cellulose (HPC). The viscous properties and *in vitro* adhesiveness increased with the amount of the polymeric additives, and retention properties of OB in rabbit bladder were comparable after addition of 0.4% HYA and 1.0% HPC. HYA was able to enhance the intravesical retention properties of OB instillation solution to a lesser degree than HPC, it seemed to be a useful additive in the OB instillation due to its safety and mucosal-protective effect.

**Key words** intravesical instillation; bladder; oxybutynin; sodium hyaluronate; viscosity; adhesiveness

For spastic neurogenetic bladder, drug therapy based on anti-cholinergics, which suppresses abnormal contraction of the detrusor muscle, has been proposed. Sympathomimetic drugs are also administered to enhance flow resistance in the urethra. However, oral administration of anti-cholinergics often induces side effects such as dry-mouth or constipation, and doses are inevitably lowered for patients with intractable urinary incontinence.

In urological fields, intravesical instillation, in which anti-tumor agents are administered through a urethral catheter, is a common therapy for superficial bladder cancers.[1,2] Brendler *et al.* also reported intravesical instillation therapy with oxybutynin (OB) hydrochloride,[3] an anti-cholinergic, in neurogenetic bladder, and the clinical usefulness of the therapy has been demonstrated by other research groups.[4—7] Enhanced effectiveness resulting from direct action on the bladder smooth muscle, and reductions in general side effects are advantages of intravesical instillation of drug solution. However, there is concern about a most OB may be discharged at the first catherization after instillation, the prolongation of the drug retention on bladder wall is required.

Recently, large variety of bioadhesive polymers are available. In the urological field, the application of bioadhesive polymers such as chitosan, polycarbophil,[8,9] poly(methylidene, alonate 2.12)[10] and hydroxypropylcellulose (HPC) have been reported.[11,12] With regard to the OB intravesical instillation, Chiba *et al.* demonstrated that addition of 1.0% HPC effective prolongs OB retention in the bladder.[13] To date, significant adverse effects regarding HPC have not been reported. However, it is important for pharmacists to consider and prepare the alternative bioadhesive polymers for any unexpected adverse effect such as allergy.

In this study, we focused attention on hyaluronic acid as other bioadhesive substance. Hyaluronic acid is a muco-polysaccharide that is present in the skin, umbilical cord, synovial fluid, vitreous body, lung, liver and kidney, and has recently attracted attention in various fields. In medical fields, hyaluronic acid has been applied as an intra-articular injection for osteoarthritis, an adjuvant for ophthalmic operations and as a wound cover on the skin. Takahashi *et al.* reported that sodium hyaluronate (HYA) effectively promotes epithelial healing of the vesical mucosa and vesical fibrosis in the bladder of rabbits with acetic acid-induced cystitis.[14] Boucher *et al.* demonstrated that HYA inhibits bladder mast cell activation, as well as the secretion of proinflammatory mediators induced by immobilization stress.[15] Due to the possible mucosal protection effect of HYA, direct instillation of sterile HYA solution into bladder has been approved as a treatment for interstitial cystitis in Canada and the European Union.

Iavazzo *et al.* also reviewed the available data regarding the use of hyaluronic acid as an alternative treatment of interstitial cystitis, recurrent urinary tract infections and hemorrhagic cyctitis,[16] and demonstrated that no serious local or systemic adverse effect was reported in the reviewed studies. More recently, HYA has been investigated as a drug delivery agent ophthalmic,[17,18] nasal[19] and pulmonary[20] routes. Furthermore, HYA has been applied as bioadhesive agents, *e.g.*, Dollo *et al.* demonstrated that the addition of the hyaluronic acid prolonged the epidural bupivacaine effect in rats.[21]

In the present study, to prolong OB retention on bladder mucosa, we attempted to prepare the intravesical instillation solution consisting of OB and HYA. The objective of this study was to investigate the effect of HYA addition on the physicochemical properties of intravesical instillation solution *in vitro* and evaluate the feasibility of its pharmaceutical utility.

## Experimental

**Materials** OB and HYA (derived from cockscomb) were purchased from Sigma Aldrich Japan K.K. and Seikagaku Co. (Japan), respectively. HPC, potassium dihydrogen phosphate·2H$_2$O, dipotassium hydrogen phosphate·12H$_2$O (Wako Pure Chem. Ind., Ltd.), sodium chloride (Tomita Pharm. Co., Ltd.) and phosphoric acid (Kanto Chemical Co., Inc.) were used without further purification. Water-for-injection was of JPXV grade.

**Preparation of OB Solutions** A fixed weight (0.5 g) of OB crystals, various weights (2—10 g) of HYA on HPC and 5.8 g of sodium chloride were added and dissolved in 1000 ml of phosphate buffer. The formula of the OB solution used in this study is shown in Table 1. The pH and osmotic

Table 1. Component of OB Solutions

| Component | OB–buffer | –0.2% HYA | –0.4% HYA | –1.0% HPC |
|---|---|---|---|---|
| Oxybutynin (g) | 0.50 | 0.50 | 0.50 | 0.50 |
| NaCl (g) | 5.80 | 5.80 | 5.80 | 5.80 |
| $NaH_2PO_4 \cdot 2H_2O$ (g) | 6.84 | 6.84 | 6.84 | 6.84 |
| $Na_2HPO_4 \cdot 12H_2O$ (g) | 2.19 | 2.19 | 2.19 | 2.19 |
| HYA (g) | | 2.00 | 4.00 | |
| HPC (g) | | | | 10.00 |
| Distilled water | *q.s.* | *q.s.* | *q.s.* | *q.s.* |
| Total (ml) | 1000 | 1000 | 1000 | 1000 |

Table 2. Nutrient Solution (Modified Krebs Solution)

| Component | Weight or volume |
|---|---|
| NaCl | 6.4 g |
| $NaHCO_3$ | 2.1 g |
| Glucose | 1.0 g |
| 0.46 M KCl | 10 ml |
| 0.25 M $CaCl_2$ | 10 ml |
| 0.12 M $MgSO_4$ | 10 ml |
| 0.12 M $KH_2PO_4$ | 10 ml |
| Distilled water | *q.s.* |
| Total | 1000 ml |
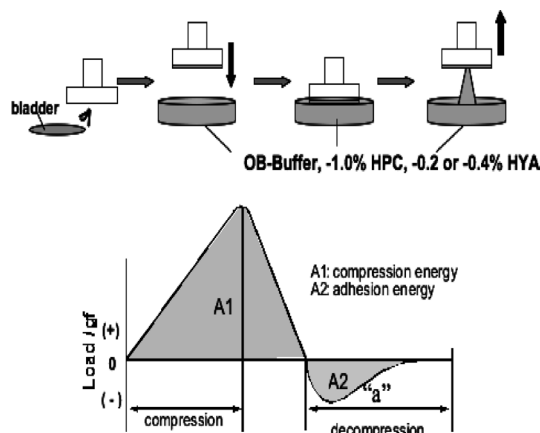


Fig. 1. Schematic View of Measurement of OB Solution Adhesiveness



Fig. 2. Hydrolysis Rate Constants in Solutions at Various pH Levels

pressure were 5.87 and 298 mOsm, respectively. The concentration of HYA was fixed at 0.2 and 0.4%, based on the tribological properties at catheterization. A solution containing 1.0% HPC was also prepared for comparison.

**Measurement of OB** OB concentration in the sample solution was determined by high-performance liquid chromatography (HPLC). HPLC apparatuses consisted of detector, SPD-10AVP; column oven, CTO-10AVP; calculator, C-R8A (219 nm; Shimadzu Co., Kyoto, Japan). Measurement conditions were as follows: column, Shodex C18M-4D (4.6 mm i.d.×150 mm; Showa Denko Co., Ltd., Tokyo, Japan); column temperature, 30 °C; mobile phase, 50 : 50 acetonitrile : 0.01 M sodium phosphate buffer (pH 3.5); elution rate, 1.5 ml/min. Data were fitted to a least squares linear regression, which gave linearity for the standard as $r>0.995$. The limit of detection (LOD) and limit of quantitation (LOQ) were 0.066 and 0.201 $\mu$g/ml, respectively.

**Chemical Stability of OB Solution** After complete dissolution in methanol, OB was introduced into phosphate buffers of various pH (pH=1, 3, 6, 8, 10, 11, 12), and OB concentration was fixed at 0.1 mg/ml. Sample solutions were then incubated in a water bath at 60 °C, and the remaining OB was determined by HPLC.

**Measurement of Viscosity** Viscosity was measured at 37±1 °C on a RE80 viscometer (Toki Sangyo Co., Ltd., Japan) equipped with a standard cone (cone angle, 1°34′; diameter, 48 mm).

**Evaluation of Intravesical OB Instillation Solution Adhesiveness** The apparatus and procedure are schematically illustrated in Fig. 1. Male Japanese white rabbits (weight, 2.4—2.7 kg) received a single ear-vein injection of 50 mg/kg pentobarbital. The bladder was extracted after median section under anesthesia, and was immersed in the nutrient solution listed in Table 2.

Adhesiveness of the instillation solutions was evaluated on an RE2-33005S Creep-meter (Yamaden, Japan) at 20 °C and 60% RH. Rabbit bladder skin in sheets was stuck to the 55 mm$\phi$ plunger surface, and the plunger level was lowered onto the surface of the sample solution. After loading down at a velocity of 1 mm/s until 10.0% strain, the plunger was pulled up until separated from the solution. The tension for separation was considered to be the adhesion force, and the distance from the solution surface at separation was estimated as an index of spinnability.

**Evaluation of OB Retention Properties in Rabbit Bladder** A 14 Fr Nelaton's catheter (Terumo Co., Japan) was connected to the extracted bladders through a TPX tube connector I-type (Sanplatec Co., Ltd., Japan). The inner volume of the catheter and the connector was fixed at 1 ml by adjusting the catheter length. The bladder was soaked in nutrient solution throughout the experiment.

After 2 ml of OB–buffer, OB–0.4% HYA or OB–1.0% HPC solution was injected into the bladder through the catheter, 1 ml of air was introduced to displace residual solution from the catheter. Buffer solution (Table 2, 30 ml) was introduced at an injection rate of 1 ml/min with a syringe pump through a new catheter, and was drawn using a 50 ml syringe. The amount of OB remaining in the bladder was estimated by determining OB concentration in the buffer by HPLC.

**Statistical Analysis** All results are presented as the mean±S.D. The significance of difference was analyzed by the use of the paired *t*-test, and a significance level of less than 5% was considered significant.

## Results and Discussion

**Determination of Optimal pH for OB Instillation Solution** The chemical stability of OB was investigated at various pH levels. The degradation of OB appeared to follow apparent first-order kinetics at all pH values. The apparent hydrolysis rate constants were obtained from individual semilogalithmic plots of remaining sample concentration *versus* time, and the apparent first-order rate constants were obtained by the least squares method. The pH-rate profile indicated that OB was stable under acidic or neutral conditions, while under alkaline conditions (pH >10), hydrolysis readily occurred (Fig. 2). These results were in agreement with those previously observed by Miyamoto *et al.*[22] Taking into account the irritation properties during intravesical use, as well as OB stability, the pH of the instillation should be 5.5—6.0.

**Adhesive Properties of OB Solution** Figure 3 shows the rheograms obtained from measurement with the rotational viscometer for buffer, oxybutynin–HYA solutions and oxybutynin–1.0% HPC solution. The apparent viscosity (mPa·s) of sample solutions were expressed as a product of the shearing stress (Pa) and the reciprocal of the rate of shear (s$^{-1}$). The order of apparent viscosity at the definite shear rate was OB–0.4% HYA>OB–1.0% HPC>OB–0.2% HYA>OB–buffer.

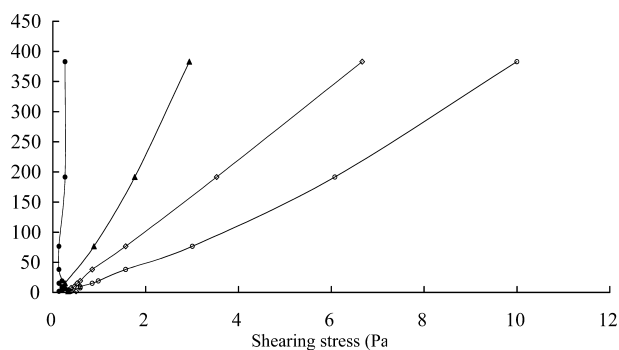Sustained action of an instillation preparation is an advan-

Fig. 3.

Fig. 3.   Flow Curves for OB Solutions

(●) OB–buffer, (▲) OB–0.2% HYA, (◇) OB–1.0% HPC, (○) OB–0.4% HYA. Each point represents the mean±S.D. (*n*=5).
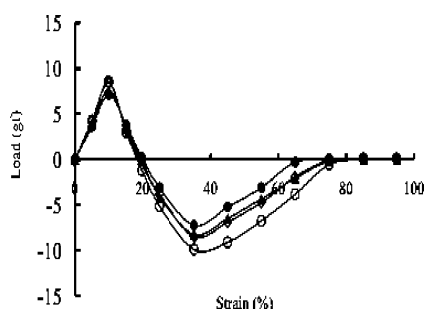


Fig. 4.   Adhesive Force–Displacement Curves of OB Solutions

(●) OB–buffer, (▲) OB–0.2% HYA, (○) OB–0.4% HYA, (◇) OB–1.0% HPC. Each point represents the mean±S.D. (*n*=5).

tage for direct inhibition by OB in bladder smooth muscle. Taking into account the practical use, the adhesion properties of OB solutions seemed to be an indication of the retention of OB.

To investigate the effects of HYA addition on the adhesiveness of OB instillation solution, the adhesive energy and spinnability of the preparations were evaluated *in vitro* by load–strain analysis on a creep-meter. Load–strain curves for each preparation are shown in Fig. 4. The highest load points for all preparations were the values at a strain of 10%, as the pulling up process of the plunger started at a loaded state of 10% strain. At the curve below the *x*-axis, the value of the load represents the tension received by the plunger, and the peak area of the curve indicates the adhesive energy between the surface of the rabbit bladder mucosa and that of OB solutions; the larger the area of the load–strain curve, the higher the adhesion energy of the sample solution. At the same time, distance from the solution surface at negative load, corresponding to part "a" shown in Fig. 1, was considered to be an index of spinnability; the higher the strain, the larger the spinnability of the sample solution. Spinnability is a rheological parameter which characterizes the property of fluids to be drawn into threads when streched.[23]

The results shows that the adhesive energy of the preparation increased with HYA concentration and the adhesiveness of the OB–0.4% HYA preparation was higher than that of the OB–1.0% HPC preparation.

On the other hand, spinnability also increased with HYA concentration, and the spinnability of the OB–1.0% HPC

Table 3.   Adhesion Energy and Displacement of OB-Solutions

| | Adhesion energy (J/m³) | | Spinnability (%) | | |
|---|---|---|---|---|---|
| OB–buffer | 7.62±0.11 | ⎤* | 46.58±1.03 | | |
| OB–0.2% HYA | 10.34±0.40 | ⎥* | 51.76±0.19 | ⎤* | |
| OB–0.4% HYA | NS 13.48±0.89 | ⎦* | 57.10±0.69 | ⎥* | * |
| OB–1.0% HPC | 10.42±0.04 | | 50.18±0.25 | ⎦ | |

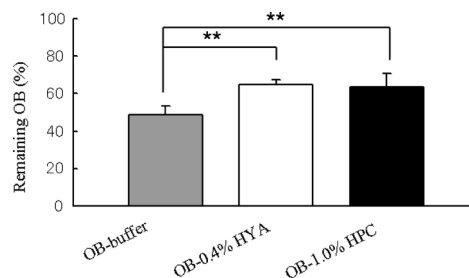Data are expressed as means±S.D. where *n*=5. *p<0.005. NS: not significant.



Fig. 5.   Amounts of OB Remaining in the Bladder

Each point represents the mean±S.D. (*n*=5). **p<0.01.

preparation was lower than that of OB–0.2% HYA (Table 3). Shin *et al.* demonstrated that the spinnability gives an indication of inner structure of polymers, *i.e.*, the polymers with a linear structure show the spinnability, on the other hand, the polymers with a 3-dimensional structure do not show the spinnability.[24] Regarding HYA, known as a linear polysaccharide,[25] it is contemplated that no inner structural changes such as gelation were occurred in the range concentration of HYA investigated in this study.

**Retention Properties of OB Solution in Rabbit Bladder** The visco-mechanical study above suggested the superiority of HYA over HPC, a conventional adhesive agent. To evaluate the effects of HYA addition on the prolongation of OB retention on bladder wall, the retention properties of OB in rabbit bladder were compared between OB–0.4% HYA and OB–1.0% HPC preparations. Figure 5 shows the percent of OB remaining in the bladder for the OB–buffer, OB–0.4% HYA and OB–1.0% HPC preparations. Preparations containing adhesive polymers clearly showed better OB retention than the OB–buffer solution, but no significant differences were seen between the OB–HYA and OB–HPC preparations.

In order to wash out the remaining OB in the bladder, the introduction of 30 ml of buffer into the bladder followed by drawing was repeated three times. OB concentrations in all the buffers drawn from the bladder were found to be below detection limits. This suggests that OB is absorbed to some extent into the bladder tissue. Grabnar *et al.* demonstrated that chitosan and polycarbophil applied to intravesical drug delivery increase permeability of the bladder wall.[9] Although in this study there was no significant difference in the OB retention property between OB–0.4% HYA and OB–1.0% HPC in spite of the difference in the adhesion energy in rheological study, this seems to be attributed to a difference in the diffusion behavior of OB by the difference of viscoelastic properties of HYA or HPC. We believe further detailed investigation of the absorption of OB will reveal ways to explain the phenomena observed in this study.

In conclusion, addition of an adhesive polymer, HYA or

HPC, clearly enhanced both adhesion force of the instillation solution and OB retention in the rabbit bladder. On the creep-meter, the OB–0.4% HYA solution had a higher adhesive force and spinnability than the OB–1.0% HPC solution, and thus HYA is thought to be superior to HPC with regard to adhesiveness to the bladder mucosa. However, no significant differences between HYA and HPC were seen in the study on OB retention in rabbit bladder. With regard to retention properties, OB sorption behavior in bladder tissue should be taken into account, in addition to physicochemical properties such as adhesiveness. Even though the HYA is still costliness in Japan and the retention properties were comparable between HYA and HPC, HYA is feasible for clinical applications due to its confirmed safety and possible mucosal protection effect.

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