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Influence of chitosan and polycarbophil on permeation of a model hydrophilic drug into the urinary bladder wall

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Abstract

Influence of dispersions of mucoadhesive polymers chitosan and polycarbophil on permeability properties of urinary bladder was investigated in vitro on isolated porcine urinary bladder. Pipemidic acid as a model hydrophilic drug was used. Its distribution in the bladder wall was determined from actual tissue concentrations by a method based on sectioning of frozen tissue and extraction of tissue slices. Pipemidic acid tissue concentration versus tissue depth profiles were evaluated by a diffusion model assuming constant diffusion coefficient. Increase in bladder wall permeability was observed in the presence of both polymers. Apparent permeability (mean ± S.D.) of urinary bladder wall was increased 2.7 ± 2.9 and 2.8 ± 2.0 times for chitosan, and 2.3 ± 2.0 and 4.3 ± 4.2 times for polycarbophil at 0.5 and 1.0%, w/v polymer concentration, respectively. This increase is a consequence of the increased permeability of urothelium. These findings support investigations on application of chitosan and polycarbophil in development of mucoadhesive intravesical drug delivery systems. Experimental model may be applied to evaluate the results of experiments with drugs used in intravesical therapy.

Keywords: Chitosan; Polycarbophil; Permeation enhancement; Mucoadhesion; Urinary bladder; Intravesical instillation

1. Introduction

Intravesical instillation of a drug solution is a common way of treating superficial bladder cancer (Witjes, 1997; Nseyo and Lamm, 1996) and, in catheterized patients, also urinary bladder infections (Getliffe, 1996; Gubbins et al., 1999). Due to the anatomical and physiological characteristics of the lower urinary tract there are several advantages of the local over systemic drug administration. As with other regional approaches intravesical pharmacotherapy aims to optimize drug delivery near the site of action while minimizing systemic exposure (Highley et al., 1999). The current approach is instillation of a drug solution using an empirical dosage regimen. To optimize treatment parameters Wientjes (Wientjes et al., 1993) developed a mathematical model to predict drug exposure in bladder tumors and to correlate the exposure with antitumor effect. The model was based on pharmacokinetic data in patients treated with intravesical mitomycin C, drug penetration data in the bladder wall of patients undergoing cystectomy and on data on the chemosensitivity of the patient's bladder tumor. By the use of computer simulation he assessed that 8.5-fold increase in tissue exposure can be achieved by dosage regimen optimization compared to standard treatment. This would result in a 20% improvement in recurrence-free rate.

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Drug concentrations in the bladder tissue are frequently subtherapeutic due to short retention times in the bladder lumen, continuous dilution as a result of urine formation, low permeability of the bladder mucosa and systemic absorption in the lamina propria and underlying muscular layers (Highley et al., 1999). Prolongation of drug retention near the site of action and maintenance of relatively constant drug concentration by controlled drug delivery are the main motives for developing an intravesical drug delivery system (Johnson et al., 1989; Frangos et al., 1990; Singh et al., 1996; Ueda et al., 1992, 1994). There is an immense potential in mucoadhesion (Bogataj et al., 1999).

In the last decade, considerable attention has been focused on the mucoadhesive polymers chitosan (CH) and polycarbophil (PC). In addition, it has been demonstrated, "in vitro" as well as "in vivo", on intestinal, bucal and nasal mucosa, that both polymers enhance permeability by modulating paracellular transport pathways. The mechanism involves epithelial tight junctions and is calcium-dependent (Schipper et al., 1997; Kriwet and Kissel, 1996; Lueßen et al., 1996). We recently showed for PC (Kerec et al., 2002) that probably the same calcium-dependent mechanism exists also in the urinary bladder. It is anticipated that utilization of CH and PC in intravesical therapy would improve efficacy by a combination of mucoadhesion and permeation enhancing effects.

To test this concept in intravesical delivery, PC and CH were used as mucoadhesive polymers, and pipemidic acid (PPA) as a model hydrophilic drug. The permeation enhancing effect was determined in isolated porcine urinary bladder wall. Furthermore, a mathematical model was developed on the basis of obtained results to get an insight into the mechanisms of permeation enhancement and to explore the potential benefits of the use of CH and PC in intravesical therapy.

2. Materials and methods

2.1. Chemicals

PPA trihydrate was provided by Lek, d.d. (Ljubljana, Slovenia). Chitosan hydrochloride—Protasan CL213 was purchased from Pronova (Oslo, Norway). According to the certificate of analysis, degree of deacetylation was 86%. Polycarbophil—Noveon AA1 was a gift from BF Goodrich (Brecksville, USA). For determining PPA in tissue samples, chromatography grade methanol (MeOH) and acetonitrile (ACN) (Merck, Darmstadt, Germany) and analytical grade trichloroacetic acid (TCA) (Kemika, Zagreb, Croatia) were used. Phosphate-buffered saline (PBS) was prepared according to European Pharmacopeia. Before use it was saturated with 95% O_2/5% CO_2 and pH adjusted to 7.4. All chemicals used were of analytical grade.

2.2. In vitro permeation study

Porcine urinary bladders were obtained from the local slaughterhouse. They were washed and transported to the laboratory in PBS at 5 °C. Previous experiments using electron microscopy showed that bladder epithelium remained intact when bladders were kept under these conditions for up to 5 h (Burjak et al., 2000). All experiments were performed within this period.

PPA distribution in the bladder wall was determined from actual tissue concentrations. For this purpose, a method to determine drug concentration-depth profiles was developed. Permeation of PPA into the bladder wall was studied in custom-designed diffusion cells (Kerec et al., 2002). Each cell consisted of two sides between which bladder wall was sandwiched horizontally. The upper side of the cells had a 10 ml donor chamber exposed to the tissue surface. Tissue exposure area was 4.5 cm^2. Three pieces, approximately 3 cm × 3 cm in size, were carefully dissected from the central part of the bladder corpus and laid with the mucosal side facing the donor chamber. The latter was filled with a solution (80 or 160 mg/l) of PPA (control experiment) or a dispersion of CH or PC (0.5 or 1%, w/v) in the same solution of PPA for 25, 50 or 120 min. All experiments were performed in at least five replicates under four different experimental conditions, which are summarized in Table 1.

In subsequent steps, a modification of the method of Wientjes was employed (Wientjes et al., 1991). Each piece of the bladder wall was placed between two parallel stainless steel plates 5 mm apart and rapidly frozen in liquid nitrogen, resulting in frozen pieces of tissue with flat surfaces. Segments of approximately 7 mm × 7 mm were trimmed from the central part of

Table 1
Experimental conditions used in the study of diffusion of pipemidic acid (PPA) in the urinary bladder wall

Experiment	PPA (mg/l)	Polymer (%, w/v)	Duration (min)
Experiment 1	80	0.5	50
Experiment 2	80	0.5	120
Experiment 3	160	1.0	50
Experiment 4	160	0.5	25

All experiments were performed at four different experimental conditions to study the influence of PPA concentration, polymer concentration and duration of exposure simultaneously.

the frozen tissue and attached by the serosal sides to cryotome object holders with Cryomatrix cryoadhesive (Shandon/Life Sciences Int., Astmoor, UK). Care was taken that cryoadhesive was applied only between tissue segments and the object holder and that edges of the tissue segments were not contaminated. The mucosal surface of the tissue segments was aligned exactly parallel to the cutting surface of the cryotome (Cryocut E, Reichert-Jung/Cambridge Instruments, Keene, USA). The segments were sectioned in 20-μm thick slices parallel to the mucosal surface to the depth of 1200 μm. Three tissue slices were pooled, placed in preweighed 1.5 ml centrifuge tubes, and their weight determined (approximately 3.5 mg). Samples were stored in their centrifuge tubes at −20 °C until assayed.

2.3. HPLC analysis

To the weighed samples of bladder tissue 400 μl of the mobile phase (0.2% TCA/MeOH/ACN, volume ratio 76/4/20) was added for PPA extraction. Samples were wortexed until all tissue slices were freely floating, shaken for 2 h to ensure complete extraction and centrifuged (10 min at 45,000 × g). Samples (20 μl) of clear supernatant solution were injected without any further preparation. HPLC analysis was carried out on PRP-1 column (Hamilton, Reno, USA) at a solvent flow of 1 ml/min using fluorescence detection at λ_{Ex} = 275 nm and λ_{Em} = 440 nm (LC 540, Perkin-Elmer, Beaconsfield, UK). Retention time was 4 min. PPA concentrations were determined using external standards. Limit of detection was 0.005 mg/l and linear range was up to 2 mg/l.

2.4. Data analysis

Bladder tissue PPA concentrations at 20 tissue depths were expressed as an amount of PPA (μg) per gram of tissue. Concentrations versus depth profiles were constructed. In all experiments, an estimated maximum amount of PPA in bladder tissue was less than 8% of the initial amount in the donor chamber of the diffusion cell. Eq. (1) was fitted to individual concentration profiles, where $C_{x,t}$ is the tissue PPA concentration in depth x at time t, C_L the PPA concentration in the luminal solution, K reflects PPA bladder tissue to luminal solution partitioning and D is PPA diffusion coefficient in the tissue.

$$C_{x,t} = KC_L \operatorname{erfc}\left(\frac{x}{2\sqrt{Dt}}\right) \quad (1)$$

Eq. (1) is a solution of diffusion problem assuming constant diffusion coefficient in semi-infinite medium at the following boundary and initial conditions (Crank, 1985):

$$C_{x,t} = C_0 = KC_L \quad \text{at } x = 0,\ t > 0 \quad (2)$$

$$C_{x,t} = 0 \quad \text{at } x > 0,\ t = 0 \quad (3)$$

Parameters K and D of the diffusion model were identified in Microsoft Excel by applying its complementary error function (erfc). Criteria function (Err), presented in Eq. (4) where $\hat{C}_{x,t}$ is a model output for a given set of parameters (D and K), was minimized using an Excel Add-in solver with Newton search algorithm. Deviations of the model output from experimentally determined tissue PPA concentrations $C_{x,t}$ were weighted with inverse of the variances of tissue PPA concentrations var($C_{x,t}$) to reduce the influence of experimental error.

$$\text{Err} = \sum_x \left[(\hat{C}_{x,t} - C_{x,t})^2 \frac{1}{\text{var}(C_{x,t})} \right] \quad (4)$$

Influence of both polymers at concentration 0.5 and 1.0%, w/v on permeation of PPA in the urinary bladder wall was evaluated by comparison of the identified model's parameters using t test. Permeability enhancement ratio R was calculated as a quotient of permeabilities in the presence (•) and absence (°) of the bioadhesive polymer for each experiment on

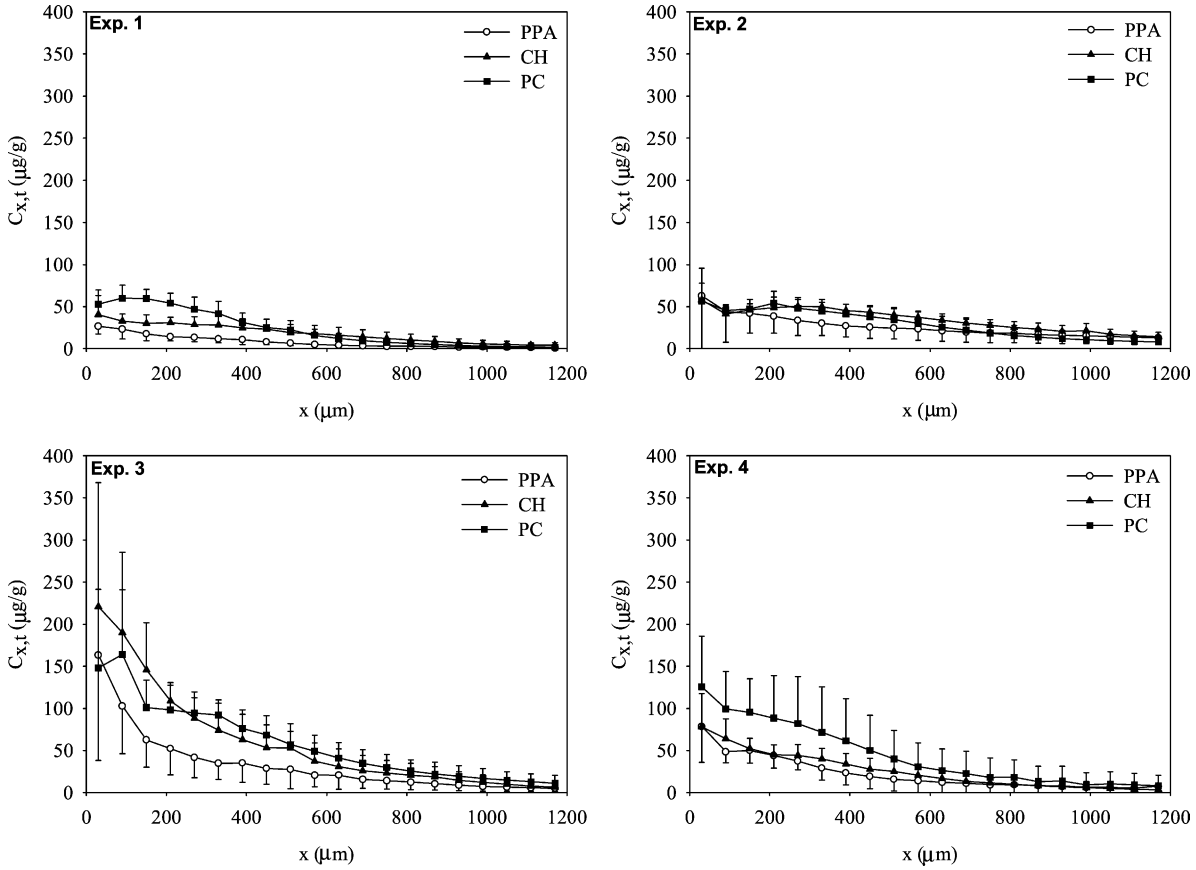

Fig. 1. Pipemidic acid (PPA) concentration vs. bladder wall tissue depth profiles (mean ± S.D.) in the presence of chitosan (CH) and polycarbophil (PC). Experiment 1: PPA 80 mg/l, polymer 0.5% (w/v), time = 50 min; Experiment 2: PPA 80 mg/l, polymer 0.5% (w/v), time = 120 min; Experiment 3: PPA 160 mg/l, polymer 1.0% (w/v), time = 50 min; Experiment 4: PPA 160 mg/l, polymer 0.5% (w/v), time = 25 min.

the same bladder according to Eq. (5).

$$R = \frac{K^{\bullet} D^{\bullet}}{K^{\circ} D^{\circ}} \tag{5}$$

3. Results

The in vitro PPA concentration versus bladder wall tissue depth at four different experimental conditions are shown in Fig. 1.

These results indicate that higher PPA concentrations are obtained in the bladder wall in the presence of CH and PC. Diffusion model (Eq. (1)) was fitted to individual concentration profiles. In general, good agreement between the model response and observed concentrations was achieved. In many cases, careful observation of the tissue concentration profiles revealed two distinct diffusion media with a breakpoint at approximately 200 μm. Steeper decrease of PPA concentration was observed in urothelium compared to tissue segments more than 200 μm below the luminal surface. There were only three experimentally determined concentrations in the superficial layer with large variability. Diffusion coefficient in the urothelium, therefore, could not be reliably estimated. Nevertheless, increased permeability of urothelium is reflected in bigger PPA bladder tissue to luminal solution partition coefficient.

The influence of CH and PC on PPA diffusion in the urinary bladder is summarized in Table 2. No

Table 2
Influence of chitosan (CH) and polycarbophil (PC) on pipemidic acid (PPA) diffusion in the urinary bladder wall (mean ± S.D.)

Solution	*n*	*K*	*D* (10^{-5} cm^2/s)
Control/PPA	31	0.37 ± 0.20	2.6 ± 2.1
CH 0.5%, w/v	16	0.56 ± 0.16*	3.2 ± 1.8
CH 1.0%, w/v	5	1.16 ± 0.24*	1.5 ± 0.9
PC 0.5%, w/v	15	0.83 ± 0.23*	2.1 ± 1.7
PC 1.0%, w/v	5	0.96 ± 0.20*	2.6 ± 2.0

n: Number of replicates; *K*: PPA bladder tissue to luminal solution partition coefficient; *D*: PPA tissue diffusion coefficient.
* Significantly different from control ($P < 0.05$, Student's *t*-test).

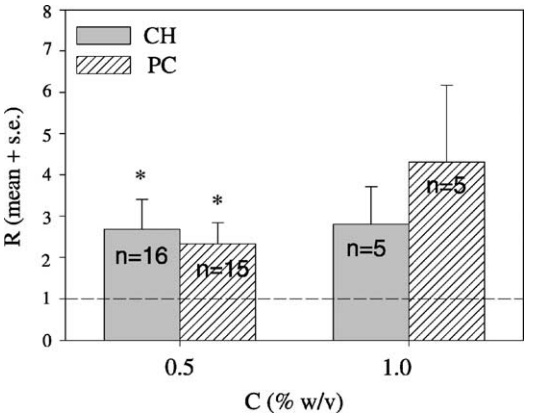

Fig. 2. Urinary bladder permeability enhancement ratio (R) of chitosan (CH) and polycarbophil (PC). (*) Significantly different from 1 ($P < 0.05$, one sample *t*-test).

significant ($P > 0.05$) impact of exposure time and PPA concentration on the PPA bladder tissue to luminal solution partition coefficient and diffusion coefficient (*K* and *D*) was demonstrated by ANOVA. Parameters determined under various experimental conditions were therefore pooled to get more reliable estimates, which are given in Table 2.

The influence of polymers on permeability of urinary bladder wall for PPA is shown in Fig. 2.

4. Discussion

Intravesical chemotherapy is widely used for more than 40 years in patients with superficial bladder cancer as adjuvant to surgical removal. It aims to optimize drug delivery in the tumor and its vicinity and reduce systemic toxicity. Commonly applied agents are mitomycin C, thiotepa, etoglucid, doxorubicin, bacilli Calmette-Guérin and recently taxol (Highley et al., 1999). Although there is sparse information on the uptake from the bladder lumen, models for drug distribution in the bladder wall and systemic absorption have been developed that led to optimal intravesical treatment regimens (Wientjes et al., 1993). Simple diffusion and distributed models have been proposed. The bladder wall is composed of roughly 200 μm thick unperfused urothelium, which is covered with highly negatively charged glycosaminoglycans and capillary perfused connective tissue—lamina propria and muscle layers (Hurst and Zebrowski, 1994; Liebhold et al., 1995). Investigations of the basis of blood–urine barrier revealed that urothelium plays a crucial role by two mechanisms. The first involves the glycosaminoglycan layer (Hurst and Zebrowski, 1994) and apical membranes, which exhibit a unique structure, with 70–90% of the area occupied by rigid appearing plaques containing paracristalline arrays of uroplakins (Chang et al., 1994). The second mechanism that contributes to low permeability is urothelial tight junctions.

In addition to the low permeability of the bladder mucosa, drug concentrations in the bladder wall are frequently subtherapeutic due to short retention of the drug in the bladder lumen and systemic absorption. The first and the second of these three obstacles may be diminished by involvement of the principles of mucoadhesion. Not only that it renders prolongation of the drug in the bladder lumen possible, it is also potentially effective by the maintenance of high drug concentrations in the immediate vicinity of urothelium and therefore increasing concentration gradient. Ueda (Ueda et al., 1994) successfully applied hydroxypropylcellulose in intravesical chemotherapy.

Mucoadhesive polymers CH and PC are well-known intestinal, bucal and nasal absorption enhancers. They modulate paracellular transport pathways by opening epithelial tight junctions (Schipper et al., 1997; Kriwet and Kissel, 1996; Lueßen et al., 1996). We tested this concept for intravesical drug delivery by in vitro experiments on isolated porcine bladders. A modification of the method based on sectioning of frozen tissue developed by Wientjes (Wientjes et al., 1991) was applied. Diffusion model based on Fick's laws

was used for evaluation of results. The model can be extended by the process of systemic absorption in lamina propria and muscular layers to simulate in vivo situation. There is a simple rule governing capillary removal of the drug that a fraction of the drug proportional to tissue–blood concentration gradient is removed each time the drug passes a capillary. Parameters of the single-phase diffusion process could only be identified by reasonable accuracy, as there were only three experimentally determined drug concentrations in urothelium. Bladder wall was therefore treated as semi-infinite diffusion medium with constant diffusion coefficient. Even though PPA concentrations in the urothelium were consequently under-predicted, it seems reasonable to assume that conclusions drawn were not affected. No significant increase in diffusion coefficient was observed in the presence of polymers as indicated in Table 2. Evidently only diffusion of PPA in the urothelium is enhanced in the presence of both polymers, while diffusion in the underlying layers of connective and muscular tissue remains unaffected. This is manifested in parameter K, which reflects PPA bladder tissue to luminal solution partitioning. In the presence of both polymers, significant increase in K was observed ($P < 0.05$, Student's t-test). Apparent permeability (mean ± S.D.) of urinary bladder wall is increased 2.7 ± 2.9 and 2.8 ± 2.0 times for CH and 2.3 ± 2.0 and 4.3 ± 4.2 times for PC at 0.5 and 1.0%, w/v polymer concentration, respectively. This increase is a consequence of the increased permeability of urothelium for both polymers at both concentrations (Table 2).

These results support further investigations on application of CH and PC in intravesical drug delivery.

5. Conclusion

CH and PC increase permeability of the bladder wall by diffusion enhancement in the urothelium. The proposed experimental model may be applied to evaluate the results of experiments with drugs employed in intravesical therapy.

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