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Colon Delivery of Prednisolone Based on Chitosan Coated Polysaccharide Tablets

Hyun-Sun Park, Jue-Yeon Lee, Sun-Hye Cho, Hyon-Jin Baek, and Seung-Jin Lee
College of Pharmacy, Ewha Womans University, Seoul 120-750, Korea

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Colon drug delivery is advantageous in the treatment of colonic disease and oral delivery of drugs unstable or suceptible to enzymatic degradation in upper GI tract. In this study, multilayer coated system that is resistant to gastric and small intestinal conditions but can be easily degraded by colonic bacterial enzymes was designed to achieve effective colon delivery of prednisolone. Variously coated tablets containing prednisolone were fabricated using chitosan and cellulose acetate phthalate (CAP) as coating materials. Release aspects of prednisolone in simulated gastrointestinal fluid and rat colonic extracts (CERM) were investigated. Also, colonic bacterial degradation study of chitosan was performed in CERM. From these results, a three layer (CAP/Chitosan/CAP) coated system exhibited gastric and small intestinal resistance to the release of prednisolone in vitro most effectively. The rapid increase of prednisolone in CERM was revealed as due to the degradation of the chitosan membrane by bacterial enzymes. The designed system could be used potentially used as a carrier for colon delivery of prednisolone by regulating drug release in stomach and the small intestine.

Key words : Colonic drug delivery, Prednisolone, Chitosan, Cellulose acetate phthalate

INTRODUCTION

Many attempts have been made to achieve colon drug delivery that is advantageous in the treatment of colonic disease and oral delivery of proteins and drugs unstable in gastrointestinal (GI) tract (Orienti, I. *et al.*, 2001, Sinha, V. C. *et al.*, 2001, Stubbe, B. *et al.*, 2001, Yang, L. *et al.*, 2002, Yoshikawa, Y. *et al.*, 1999). A selective advantage is obtained because the colonic or rectal tissue has a greater exposure than the systemic circulation relative to that obtained by other routes or modes of administration (Tozler, T., 1990). Colon-specific drug delivery is also of special interests for compounds that are susceptible to enzymatic degradation in the upper gastrointestinal tract, such as proteins and peptides (Ghandehari, H. *et al.*, 1995). Peptide drug, like insulin can not be directly administrated orally, since they are digested by brush border peptidases before they can be absorbed (Rubinstein, A. *et al.*, 1992). Also, colon has specific enzymatic activity derived from anaerobic colonic bacteroides and has slightly higher pH values compared with that of other GI tract (Rubinstein, A. *et al.*, 1992) and colonic delivery systems has been based on the phenomena described above. Ulcerative colitis, Crohns disease, irritable bowel syndrome, diarrhea and constipation could be treated successfully if localized colonic delivery of therapeutic agents is achieved (Milojevic, S. *et al.*, 1996). Therapeutic agents utilized for the treatment colon disease include antiinflammatory agent and anticholinergic agents (Tozler, T. *et al.*, 1990). Large intestine is composed of anaerobic microflora and these microbes release their characteristic enzymes. And these microbes may affect drug metabolism with their enzymes (Rubinstein, A. *et al.*, 1992). Especially, these enzymes cleavage specifically azo-bond (azoreductase), glycosidic bond (glycosidase) and β (1→4) bond of substrates. The specificity of these enzymes resulted form the presence of biochemical processes within the colon, can convert prodrug to active drug (Tozer, T. *et al.*, 1990). Therefore, application of these enzymes derived from microorganisms in the human colon might be beneficial to improve colon drug delivery.

In this study, we developed multilayer coated system that is resistant to gastric and small intestinal conditions but can be easily degraded by colonic bacterial enzymes. For this purpose, chitosan and CAP were utilized as coating materials to design the colonic drug delivery system

Correspondence to: Seung-Jin Lee, College of Pharmacy, Ewha Womans University, Seoul 120-750, Korea
E-mail: sjlee@ewha.ac.kr

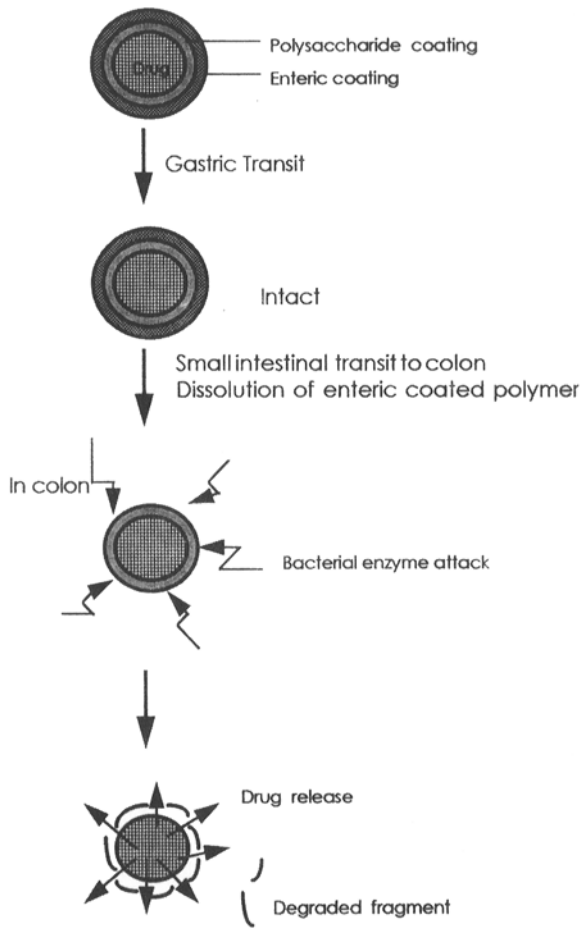

Fig. 1. The principle of the polysaccharide-enteric coated colonic drug delivery system

(Fig. 1). Chitosan is the *N*-acetylated chitin and is linear cationic polymer, having structural characteristics similar to that of glycosaminoglycans, consists of (1→4)-β-linked-2-deoxy-2-amino glucose structure (Muzzarelli, R. *et al.*, 1993, Itoli, H. *et al.*, 1985). Chitosan is valuable pharmaceutical additives due to its biocompatiblity and degradability in vivo (Sawayanagi, Y. *et al.*, 1983). Chitosan dissolves in acids with stirring to give highly viscous solutions at low concentrations and to from strong films on evaporation of the solution, and as the film is practically insoluble in higher pHs. CAP is an enteric coating material and does not dissolve until the pH of the media is higher than 6. It was reported after in vivo studies on the disintegration of CAP-coated tablets and capsules possessed satisfactory enteric characteristics (Hodge, H. *et al.*, 1994). Prednisolone widely used for the treatment of colon disease such as colitis was selected in this study as a model drug. Fabrication of multiplayer coated tablets made of polysaccharide-enteric polymers, release kinetics in simulated intestinal fluid, colonic bacterial biodegradation of chitosan using rat colonic extracts will be discussed in this paper.

MATERIALS AND METHODS

Materials

Chitosan, sodium alginate, and cellulose acetate phthalate (CAP) were purchased from Sigma Chemical Company (St. Louis, MO, USA). Prednisolone was kindly donated by Han Dok Pharmaceutical Co., Ltd (Seoul, Korea). Lactose was obtained from Ajax Chemicals (Auburn, Australia). All other reagents and solvents used were of analytical grade.

Tablet preparation

300 mg of tablets containing mixture of lactose, hydroxypropylmethyl cellulose, and prednisolone were prepared by direct compression with pressure of 7000 lbs. The prepared tablets, with diameter of 13 mm and thickness of 2 mm were coated with chitosan solution, and further coated with CAP solution by dip coating method.

In vitro drug release

Drug release studies from variously coated tablets were carried out using shaking incubator at 37°C and speed of 150 rpm. The different dissolution media used were as follows: 300 ml of artificial gastric juice with no enzyme (pH 1.2 buffer, USP) was used for 3 hrs followed by 4 hrs treatment with 300 ml of artificial intestinal fluid (pH 7.4 phosphate buffer, USP). The subsequent release tests of coated tablets were performed with 150 ml of colonic extract as releasing medium (CERM) or inactivated CERM.

The CERM was prepared by the following method. Wistar rats were sacrificed and their colons were removed. Subsequently, under anaerobic conditions, the colon was opened, and its content was suspended in 150 ml of phosphate buffered saline (PBS), which was then bubbled with nitrogen to remove the oxygen. The inactivated CERM was prepared by sterilization the CERM with autoclave. Each bottle with a rat colonal extracts was maintained in anaerobic condition for releasing period.

Each test was run in sets of four and the average amount release of drugs over time was then calculated. The concentration of predisolone in pH 1.2 was determined at 240 nm using UV-Vis spectrosphotometer (DU-68, Beckman, USA). The concentration of prednisolone in pH 7.4 and CERM was determined with HPLC (Waters 501, Milipore, USA). The condition of HPLC analysis was as follows. Column : μ-Bondapak C_{18}, flow rate : 1.0 ml/min, mobile phase : methanol:water = 60 : 40, attenuation : 32, Aufs : 0.01, sample : prednisolone in PBS, injection volume : 20, detection : UV detector at 245 nm, retention time : 7.6-8.15 min.

In vitro degradation of chitosan

To investigate the biodegradability of chitosan by colonic

bacterial enzymes, chitosan film was prepared. The weighed chitosan film were put in the each four sets of bottles with CERM or inactivated CERM and incubated under the same condition as that of release tests. Chitosan films were sampled at determined time intervals, and degraded chitosan film in CERM and in inactivated CERM were determined by weighing and calculating the remaining weight fraction.

RESULTS AND DISCUSSION

Release studies of prednisolone in artificial gastric juice and intestinal fluid

Fig. 2 demonstrates the effect of chitosan coating on the release of prednisolone in artificial gastric juice and intestinal fluid. The core tablet containing prednisolone was coated with chitosan and CAP, successively. The tactic in the design of tablets was to minimize non-specific release of prednisolone in the stomach and small intestine. No release of prednisolone was observed in pH 1.2 artificial gastric juice as expected. On changing the releasing medium to pH 7.4 artificial intestinal fluid, release of prednisolone initiated depending on coating amount of chitosan. Increase of chitosan coating weight resulted in decrease of release rate of prednisolone.

The chitosan layer could be protected from dissolution by the overlaid CAP layer in pH 1.2 medium. The change of pH of medium to 7.4 induced gelation of chitosan layer, which then functioned as a rate controlling membrane. About 20% release of loaded prednisolone was observed from the tablet coated with chitosan by 10% weight ratio to tablet during 4 hours of releasing time.

The architecture of the tablet was modified to further suppress the release of prednisolone in pH 7.4 artificial intestinal fluid. An additional CAP layer was placed under the chitosan layer, that is, a sandwiched chitosan layer between the two CAP layers (CAP/chitosan/CAP). The new CAP layer covering the core drug reservoir might induce further retardation of non-specific release of prednisolone, and the result is demonstrated in Fig. 3. The CAP/chitosan/CAP coated tablet revealed significant reduction of the reduction of the release of prednisolone. It is noteworthy that 2 hours of lag time was observed from the tablet exposed in the pH 7.4 artificial intestinal fluid. The lag time was probably due to the time for hydration of CAP sublayer surrounding the prednisolone reservoir. Simple retardation of release of prednisolone in pH 7.4 medium might be unfavorable since it also possibly causes ineffective release in the colon. However, it might be postulated that CAP will be dissolved at pH 7.4 and gradually build up hydraulic pressure within the overlaid chitosan layer in a gel state. This fact may be beneficial to facilitate the disintegration of chitosan layer by colonic bacterial enzymes and subsequent release of prednisolone.

Release studies of prednisolone in CERM

The system coated with CAP/chitosan/CAP was chosen for the release studies using CERM because it had been found to prevent release of drugs most effectively during gastrointestinal transit of tablets (Fig. 3). The release test using CERM to investigate the release mechanism of

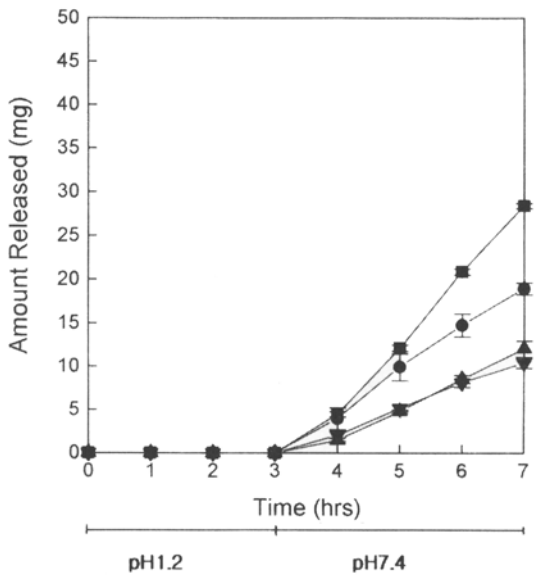

Fig. 2. Effects of chitosan coating weight on prednisolone release form coated tablets (prednisolone : lactose : HPMC = 50 mg : 125 mg : 125 mg). (■) 20% CAP, (●) 5% chito/20% CAP, (▲)10% chito/20% CAP, and (▼) 20% chito/20% CAP.

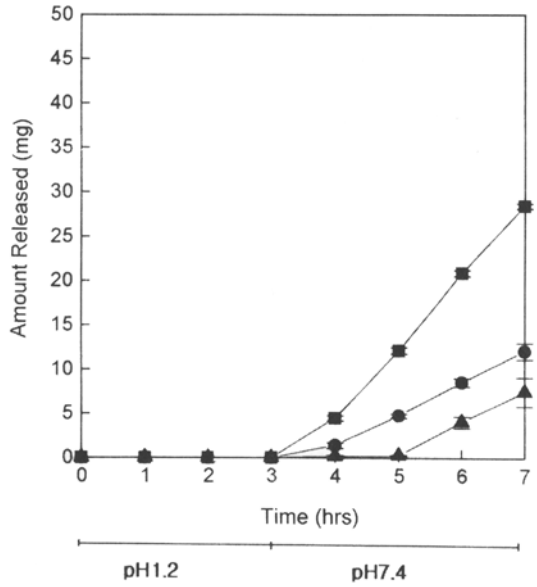

Fig. 3. Effects of coating method on prednisolone release form coated tablets (prednisolone : lactose : HPMC = 50 mg : 125 mg : 125 mg). (■) 20% CAP, (●) 10% chito/20% CAP, and (▲) 5% CAP/10% chito/15% CAP.

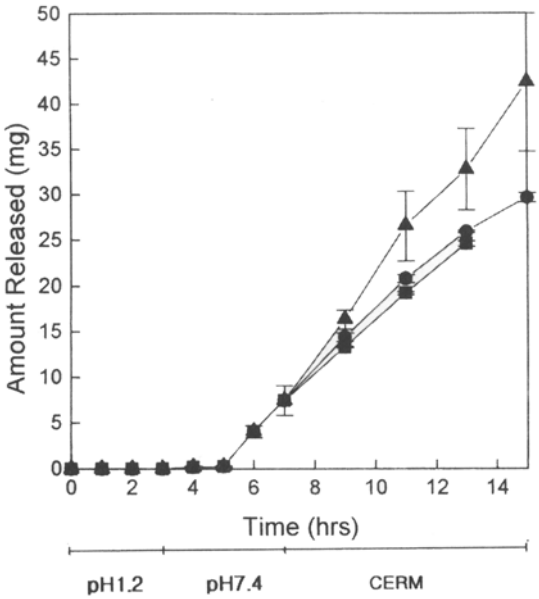

Fig. 4. Effects of activity of colonic extracts on prednisolone release form 5% CAP/10% chito/15% CAP coated tablets (prednisolone : lactose : HPMC = 50 mg : 125 mg : 125 mg). (■) pH 7.4, (●) inactivated CERM, and (▲) active CERM.

coated tablets was carried out successively following the release test with artificial intestinal fluid and the results are shown in Fig. 4. The release profiles revealed that drug release was increased rapidly in CERM compared with in the inactivated CERM and pH 7.4. The release of prednisolone was mostly occurred in CERM considering the overall releasing time course. The increase in the release rate in CERM was most probably due to biodegradation of chitosan membrane coated the tablet by colonic bacterial enzymes. A coating formulation comprising a mixture of glassy amylose and Ethocel® (ethylcellulose) was introduced as a carrier for a range of drugs requiring delivery to the colon. This system also utilized the mechanism of release by colonic bacterial degradation of the amylose domains (Milojevic, S. *et al.*, 1996).

In vitro biodegradation study of chitosan

Biodegradation of chitosan, polysaccharide with β (1, 4) bond known to be cleaved by -glycosidase, one of the colonic bacterial enzymes, was investigated. Chitosan film was degraded only 10% of original weight in case of treated with inactivated CERM. In contrast, in case of treated with active CERM, the biodegradation was increased to 40% of original weight of chitosan film (Fig. 5). There was no obvious change on the surface of chitosan film immersed in inactivated CERM. The pore and crack were formed on the surface of chitosan film treated with active CERM. This result demonstrated that chitosan can be degraded by colonic bacterial enzymes.

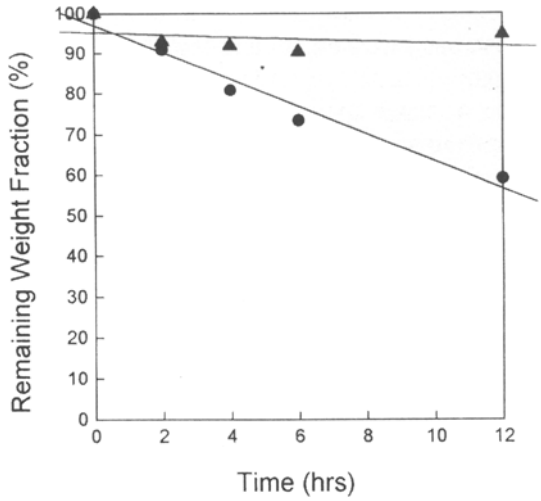

Fig. 5. Remaining weight fraction of chitosan film after in vitro biodegradation studies. (●) active CERM and (▲) inactivated CERM.

CONCLUSION

The CAP/Chitosan/CAP coated tablet exhibited gastric and small intestinal resistance in vitro most effectively. The mechanism of rapid increase of drug release in CERM was due to the degradation of chitosan membrane by bacterial enzymes The CAP/chitosan/CAP coated system might be potentially used as a carrier for colon delivery of prednisolone.

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