Colonic Drug Delivery: Enhanced Release of Indomethacin from Cross-Linked Chondroitin Matrix in Rat Cecal Content

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INTRODUCTION

Colonic drug delivery has gained in interest lately. In addition to sulfasalazine and olsalazine, colon-specific prodrugs were reported (1–3). In this report we suggest the use of a bioerodible matrix, with the ability to carry a variety of drug molecules into the colon.

Chondroitin sulfate (ChS) (Fig. 1) is a soluble mucopolysaccharide consisting of D-glucuronic acid linked to *N*-acetyl-D-galactosamide (4,5). In the human colon, the natural sources of ChS are sloughed epithelial cells and dietary meat. Chondroitin sulfate is utilized as a substrate by the bacteroids of the large intestine, mainly by *B. thetaiotaomicron* and *B. ovatus* (6,7). We suggest, therefore, that ChS could be used as a colonic drug carrier. Such use would depend on its persistence as a solid dosage form in the physiological environment of the stomach and small intestine. Since natural ChS is readily water soluble, it might not protect its load successfully. However, cross-linked chondroitin would be less hydrophilic and thus would provide a better shield. We have also assumed that cross-linked chondroitin could still be degraded by bacterial residents of the colon, to release its drug content. Therefore, cross-linked ChS may serve as a carrier for drugs targeted specifically to the large bowel.

In this study we have examined the *in vitro* degradation of ChS with varying degrees of cross-linkage in rat cecal contents. Indomethacin was used as a drug model which was incorporated into the cross-linked chondroitin matrix.

MATERIALS AND METHODS

Cross-Linking Chondroitin Sulfate. Chondroitin sulfate type A (ChS) (Sigma, St. Louis, MO) was treated with 1,12-diaminododecane (Sigma). The reaction was catalyzed by dicyclohexylcarbodiimide (DCC) (Sigma) at a 1.1:1 molar ratio of DCC to diamino reagent (8), where the ratios of ChS to the diamino reagent were 70:30, 50:50, and 40:60. The purification procedure for the cross-linked polymer was as follows: each gram of crude cross-linked polymer product was rinsed with 200 ml of acetone (analytical grade, BDH, Poole, England). One-gram samples of the rinsed product were collected and mounted in 20-cm dialysis bags (Spectra/por 6 × 30 mm; MW cut off, 12,000–14,000; Spectrum, Los Angeles). Each bag was immersed in 2.5 L of double-distilled water, which was stirred overnight at 200 rpm with a magnetic stirrer. This last procedure was repeated three times over 3 consecutive days. The product thus obtained was then placed in a 200-ml round-bottomed flask and freeze-dried overnight. Complete dehydration was validated by no weight loss on further drying. The resulting dry powder was collected and sealed till further processing. Each batch of cross-linked ChS powder was kept separately.

Qualitative IR analysis was performed after dispersing the product in KBr disks. The IR spectra were characterized by (a) the appearance of the $-CH2-$ band (2840–2900 cm^{-1}) for which the increase correlated with the amount of the diamino reactant used in the cross-linking treatment and (b) the reaction of chondroitin sulfate with the diamino reagent resulting in an increase in the typical peak of $-CONH-$ at 1600–1620 cm^{-1} (in addition to the amide group at position 2 of the galactose moiety of the untreated ChS). The degree of cross-linking was quantitatively determined by measuring the amount of methylene blue which was adsorbed as a result of cation exchange. Samples of 10 mg of natural or variously cross-linked chondroitin sulfate were mounted in a 1-cm dialysis bag (Spectra/por 6 × 30 mm; MW cutoff, 12,000–14,000; Spectrum) and immersed in 0.1% (w/v) methylene blue in Veronal buffer (Sigma) at 37°C. The methylene blue, in turn, diffused into the bag and adsorbed onto the dispersed powder. The chromophor disappearance from the outer vessel was monitored spectrophotometrically (Spectronic 1001) at 665 nm at predetermined time intervals. Equilibrium was determined when no further reduction in methylene blue concentration was noted. The difference in the absorbance for a system containing cross-linked product was divided by that obtained in the system containing untreated ChS. This value multiplied by 100 was determined as the relative methylene blue adsorption number (RMN), as follows:

$$RMN = (A_0 - A_m)/(A_0 - A_c) \times 100 \quad (1)$$

where A_m is the absorbance value of methylene blue solution at equilibrium in the system containing cross-linked ChS, A_c is the absorbance value of the methylene blue solution containing untreated ChS, and A_0 is the initial absorbance value of the methylene blue solution before any adsorption occurred.

Calculated in this way, and rounded off to the nearest unit, the RMN value for the ChS treated with diamino reagent at a ratio of 70:30 was 70 ("ChS_{70}"); for that treated at a ratio of 50:50, 60 ("ChS_{60}"); and for that treated at a ratio of 40:60, 55 ("ChS_{55}"). Thus the RMN is proportional to the water solubility of the product but is inversely proportional to the extent of cross-linkage.

The cross-linked ChS dry powder was sieved and mixed with indomethacin (Sigma, St. Louis, MO) at a ratio of 9:1

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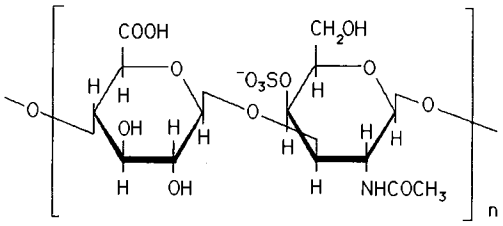

Fig. 1. Chondroitin sulfate.

(w/w). Matrices, each weighing 200 mg, were then directly compressed (3 ton), using a Perkin Elmer manual press.

Cecal Content Medium. Twenty-four hours prior to the release experiments, five Sabra rats (200–300 g) (9) maintained on a normal diet were intubated with Teflon tubing. To induce the chondroitin sulfate-lyase enzymes postulated to be in the cecum, we administered 1 ml of 20% (w/v) chondroitin sulfate aqueous solution directly to the rats' stomachs through the Teflon tubing, which was removed afterward. Thirty minutes before the drug release experiments the rats were sacrificed by an intracardiac injection of 1 ml sodium pentobarbital (6 mg/100 g body weight). The cecum was ligated in two ends (2-cm distance), cut loose, and immediately removed from the rat body. The formed cecal bag was then opened, weighed, pooled, and suspended in phosphate-buffered saline (PBS; pH 7) to give a final cecal dilution of 1.25% (w/v). As the cecum is a naturally anaerobic environment, we maintained anaerobiosis by carrying out this last step under CO_2.

Drug Release Experiments. Each drug release experiment represented a different batch of the cross-linked ChS and was performed in duplicate in 100-ml sealed glass vials which were shaken at 80 rpm in a 37°C water bath under a CO_2 atmosphere. We tested three batches each of the three levels of cross-linkage with different pools of cecal content, and for each level we reported the average of the results of the three experiments. The release experiments were performed in PBS (pH 7) with added cecal content or in PBS alone as a control. An indomethacin assay was done on 1-ml samples withdrawn in triplicate at predetermined time intervals. Indomethacin is a poorly soluble substance; therefore, proper sampling is ultimately dependent upon appropriate agitation prior the samples withdrawal. Each time, 3 ml of PBS was added back to the system to maintain constant volume and pH. The experiment was performed over 28 hr.

Indomethacin Analysis. One-milliliter samples were acidified with 200 μl 0.4 *N* HCl and extracted with 1 ml ethyl acetate containing 0.2 mg% flufenamic acid as an internal standard. The mixture was vortexed and then centrifuged for 3 min at 3400 rpm. Aliquots of 500 μl of the organic phase were evaporated, and the residue was redissolved in a 50:50 mixture of phosphate buffer, pH 7.5 and acetonitrile. Twenty microliters of the solution was injected into the HPLC system (Hewlett Packard 1050 pumping system, Jasco 875 Inteligent UV/Vis detector, Hewlett Packard 3365 ChemStation data analyzer, and Hewlett Packard analog-digital 35900C dual channel interface convertor). The wavelength was 280 nm, and the column was 5 μm, 250 × 4.6-mm RP-18 (LiChroCART 250-4, E. Merck, Germany).

Statistical Analysis. A one-way paired *t* test was done at each time point to analyze the significance of the differences in the amounts of indomethacin release in rat cecal content vs the controls. A difference was considered to be statistically significant when the *P* value was less than 0.05.

RESULTS AND DISCUSSION

Cross-linked chondroitin sulfate is degradable by bacterial enzymes in the rat cecum as demonstrated in Fig. 2. Although no clear pattern of release kinetics could be drawn, it is clear that in the ChS_{70} formulation the drug release was faster in the rat cecal medium as compared to the PBS control medium over the entire experiment and significantly higher from 12 hr on ($P < 0.05$). Salyers and co-workers (6,7) have already shown that human colonic anaerobic bacteroides can utilize chondroitin sulfate as a substrate. We have shown here that enzymes from the rat cecum, probably of bacterial origin, can also degrade chondroitin sulfate even after it has been cross-linked and its solubility reduced. The reduced solubility is essential for the resistance of solid dosage forms aimed at the colon in the upper part of the alimentary canal, where the drug release is unwanted. Thus, cross-linkage is necessary to protect the drug until it arrives in the distal part of the small intestine.

In order to analyze the specific degradation of a drug carrier it was necessary to use a water-insoluble drug model like indomethacin. Using a highly soluble drug in such a matrix system would make it hard to distinguish between simple diffusion of the drug and erosion of the drug carrier.

Table I summarizes the total amounts of indomethacin released in the rat cecal content medium at the end of each experiment (i.e., after 28 hr). The total amount of indomethacin released (i.e., when the experiment was terminated) decreases as the degree of cross-linking increases. The drug release may be controlled by balancing the cross-linkage or by using mixtures of the variously cross-linked ChS carrier in the matrices.

The enhanced release of indomethacin from the ChS_{70} formulation indicates that it may serve as a colon-specific delivery system. A matrix tablet composed of cross-linked ChS can retain 70% of its drug content for over 28 hr at pH 7 (Fig. 2), which is close to the physiological pH of the small

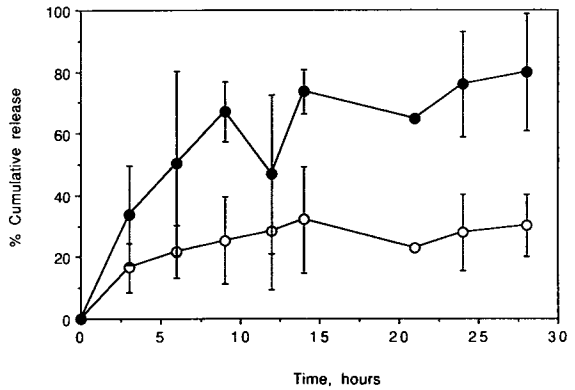

Fig. 2. Percentage cumulative amounts of indomethacin released from ChS_{70} as analyzed in rat cecal content medium (filled circles) and in PBS (open circles). Data shown are the mean of three sets of experiments. Note: The low value at 12 hr is caused by insufficient agitation of the release medium, containing the indomethacin dispersion, in one set of experiments (see Materials and Methods).

Table I. Maximum Amounts of Indomethacin Released After 28 hr from the Three Cross-Linked ChS Formulations, ChS_{70}, ChS_{60}, and ChS_{55}, in Rat Cecal Content[a]

Product	RMN value found	Maximum amount of indomethacin released after 28 hr (% of initial amount ± SD)
ChS_{70}	68.6	71.0 ± 19.0
ChS_{60}	60.5	49.0 ± 35.0
ChS_{55}	54.4	22.0 ± 7.9

[a] Data shown are the means of three sets of experiments.

intestine. On the other hand, in the presence of rat cecal content at the same pH value, enhanced release of indomethacin occurs. If extrapolation can be made to the human colon, this carrier may be used to localize drugs to the large intestine. The formulation technique presented here permits the incorporation of drugs such as steroids or salicylate derivatives like 5-amino salicylic acid, used for the treatment of inflammatory bowel diseases.

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