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Synthesis and evaluation of some polysaccharide-corticosteroide conjugates as colon specific prodrug.

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ABSTRACT

To synthesize Prednisolone-succinate-dextran.βcyclodextrin and chitosan conjugate and to evaluate the potentiality of conjugates for the treatment of inflammatory bowel diseases. Prednisolone was attached to dextran, chitosan and βcyclodextrin using succinate anhydride in an anhydrous environment catalyzed by 4-dimethylaminopyridine. The chemical structure of conjugates was identified by IR and NMR, The Prednoisolone conjugates was obtained in two steps and the in vivo drug release behavior of conjugates was investigated after oral administration of prodrugs suspension. The Prednisolone conjugates was stable in rat stomach and small intestine and negligibly absorbed from these tracts and easily hydrolyzed by an esterase. The results of this study indicate that Prednisolone conjugates may be useful in selectively delivering corticosteroids to the colon.

Keywords: Prednisolone succinate(pds),dextran(Dxt).βcyclodextrin(β-CyD)and chitosan(Cht), conjugate. 4-dimethylaminopyridine(DAMP), N-hydroxy succinimide (NHS)

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INTRODUCTION

Inflammatory bowel diseases, which include ulcerative colitis and Crohn's disease are currently treated with glucocoticoids and other anti-inflammatory agents[1,2]. For a steroidal anti-inflammatory drug, e.g. dexamethasone or prednisolone, a long-term administration would produce systemic side effects, including adreno suppression, Cushinoid symptoms, immunosuppression, and diabetes. In this case, it is desirable to localize the release of dexamethasone insofar as possible to the afflicted sites in the colon. Release of drug in the proximal GI tract should be avoided to circumvent absorption from the small intestine, and consequent drug wastage and systemic side effects[2]. Because of the unique physiological characteristics of the large intestine, drug delivery to the colon can be achieved in different ways, including pH dependent approaches utilizing the changes in pH along the GI tract[3-12], coated dosage forms[3-17], time-controlled or pulsatile release systems[18-24] pressure-controlled colon delivery systems[25], coating drugs with bacterially degradable polymer and delivery of drugs as prodrugs. The bacterial count in the colon is higher than that in the receding sections of the GI tract by many orders of magnitude in humans and other animals. Enzymes of the colonic bacteria can specifically degrade some kind of polysaccharides and azopolymers or break the chemical bonds between the parent drug and the carrier, and then the pharmacological active component can be released from natural and synthetic prodrugs. The most important issue for this approach is a selection of the functional groups that can survive the passage through stomach and small intestine, but are degraded by enzymes of the colonic microflora thus specifically releasing the drug into the colon[2].This project use prednisolone as the model drug to synthesize a prodrug via a succinate tetracarbon-bridge that links the parent drug to the dextran, chitosan and βcyclodextrin carrier. Compared with unmodified prednisolone, prednisolone conjugates are more hydrophilic and have a large molecular weight, which may decrease its possibility of being absorbed into the systemic circulation through the small intestinal epithelial cells. When it arrives to the colon, the conjugates are hydrolyzed quickly and then the esterase breaks the ester bond to release the prednisolone. .

MATERIAL AND METHOD

Prednisolone was purchased from Jubilant Pharma Delhi, 4-dimethylaminopyr idine (DMPA), and dextran, (weight-average molecular weight=70,400Dalton) βcyclodextrin and chitosan were obtained from Sigma Chemical Company,. Succinate anhydride was purchased from Lebchem.

Synthesis of Prednisolone conjugates

Prednisolone 3.98 g, succinate anhydride 1.27 g and 4-dimethylaminopyridine 1.55 g were dissolved in 400 ml .anhydrous acetone over 5Å molecular sieves. The reaction solution was stirred at 25 for 30 minutes, and the resulting solution was evaporated in a rotary evaporator to produce light yellow solid. After the solid was dissolved in anhydrous ethanol, distilled water was added to achieve a solution of ethanol and water (29:71v/v). The solution was kept at 4°**C** for 48 h to crystallize and filtered under reduced pressure. The resulting crystals were dried in a P2O5 drying pistol with refluxing of 95 % ethanol under vacuum (10 mmHg) for 24 h to produce prednisolone succinate hemiester (PDS). The yield is 85.28 4.57 %.Then in reaction scheme Appropriate prednisolone-succinate (1.151g, 0.005 M) was added to the β-cyclodextrin (5.675 g, 0.005 M) / dextran (4.520 g.005 M) /chitosan(5.675 g, 0.005 M) n 80 mL DMSO. Solution of DCC (1.031g, 0.005M) in 20 mL DMSO was added drop wise over a period of half an hour and N-hydroxy succinimide (NHS) as catalyzing amount was added. The mixture was stirred at room temperature for 72 hour, anhydrous ethanol and ether solution (100 mL 1:1) was added and the product was filtered, washed three times with ethanol to remove the byproduct dicyclohexylurea. conjugates was purified by column chromatography (DIAION® HP-20), eluting with methanol-water solution with increasing methanol contents. The elutes were monitored by TLC, and the conjugates appeared in the elutes of 30–50% methanol. After methanol was removed under reduced pressure, (TLC: silica gel) ethyl acetate-2-propanol-ammonium hydroxide-water (7:7:5:4,v/v indicator p-anisaldehyde); O-),3200.01 cm (N-H) R_f - 0.52(β-CyD- Pds)/ R_f - 0.54(Dex-Pds)$_e$ and R_f - 0.59 (Cht-Pds)

IR (β-CyD- Pds)-2962.76 cm (C-H), 1660 cm (C=C), 1267.27(-C-O-) cm,1020 cm^{-}(C-0-C) 1H NMR (500 MHz, D2O): ı (ppm) 7.57–7.59 (steroids), 5.11 (CyD, 1_-H), 5.09–5.10 (CyD, 1-H), 4.40–4.51 (steroids),3.60–4.01 (CyD, 2–6-H), 3.12-3.38 (CyD, 6_-H);

IR(Dex-Pds)$_e$ 642.32 cm (C=C bending) ,1020 cm^{-}(C-0-C), 1660 cm (C=C),1762.64(-C=O-)
1H NMR (500 MHz, D2O): ı (ppm) 7.285 (d, 1H, C-1), 6.233(d,1H,C-2), 6.002 (s, 1H,C-4), 3.488,3.508,3.623,3.742,4.668(s, 1H,C-5,C-4,C-3,C-2,C-1), 2.O54(S.2H.C-21),1.464(S, 2H,C-19), 0.860(S,3H.C-16). IR(Cht-Pds)- 1020 cm^{-}(C-0-C), 1660 cm 1H NMR (500 MHz, DMSO-d6, δ ppm) 7.35-7.33 (d, 1H,steroid), 6.16-6.14(d,1H,steroids), 4.04-3.18 (m, 6H,Chitosen),2.62-2.26(msuccinyl-)),2.00-1.78(m.3H.steroid),1.34(S,3H,steroid),0.73(S,3H.steroid),(C=C) ,1762.64(-C=

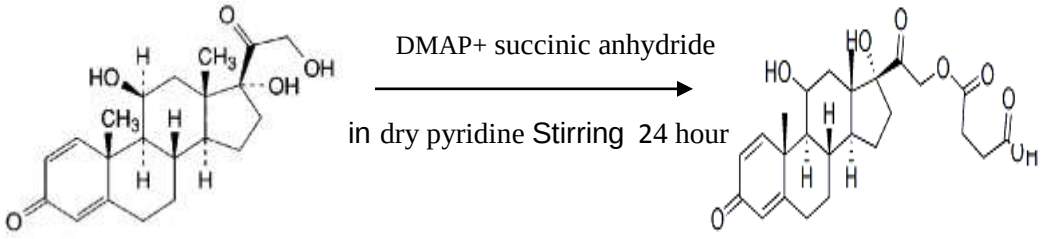
RESULTS AND DISCUSSION

Chemistry

Conjugates was prepared in two steps with a modified Mcleod reaction2]. It is essential to keep the reaction continuing under the anhydrous condition to ensure high yield. Succinate anhydride was coupled to the Prednisolone hydroxyl group in anhydrous acetone in the presence of 4-dimethylaminopyridine to produce hemiester. Then the hemiester was coupled to dextran/ β-cyclodextrin/ chitosan in DMSO using .DCC and NHS as catalyzer (Scheme 2).

The chemical structure was identified by 1HNMR and IR, confirming the procedure of scheme I and II.

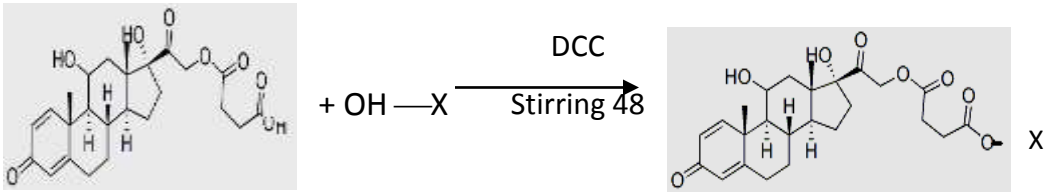
REACTION SCHEME-I



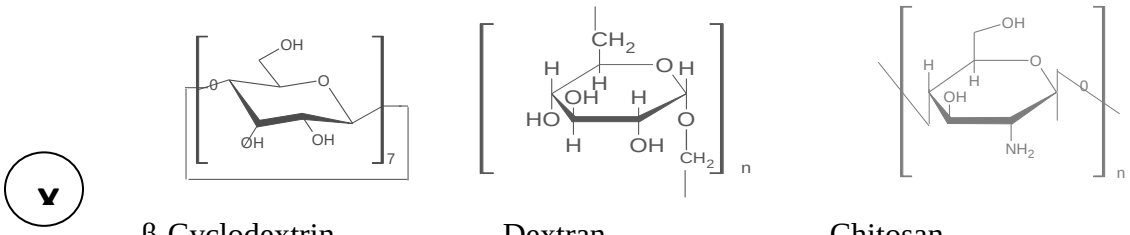
Prednisolone Prednisolone succinate

Scheme 1: represents Synthesis of Pd Succinate (PdS)

REACTION SCHEME-I I



PDS + Polysaccharides Conjugate



β-Cyclodextrin Dextran Chitosan

Scheme 2 - represents conjugation of polysaccharides with Prednisolone-succinate,β-cyclodextrin-(β-CyD-pds)e, dextran- Prednisolone-s(Dex-pds)e, Prednisolone-s -chitosan(Cht- pds)e, - having ester linkage

In vivo release study of conjugates after oral administration to rats

The Animal Ethical committee of Dr. H. S. Gour Central University, Sagar (M.P), approved the study.

Trinitrobenzene sulfonic acid – induced colitis model was used. The colonic injury and inflammation were assessed by using clinical activity score system, histopathology studies, and colon wet/dry weight ratio. The animals of test groups were given conjugates orally for eleven days and on the twelfth day one albino rat from each group was anesthetized and sacrificed 24hr after the last drug administration. An 8cm long segment of colon was excised and the section of colon was separated for wet/dry weight ratio study. To quantify the inflammation 1cm long segments was fixed in 10% formalin phosphate buffer solution (pH 7.4) for histopathological studies. Histopathological studies of the colon were carried out using haemotoxylin and eosin stains The results are shown in figure 2.

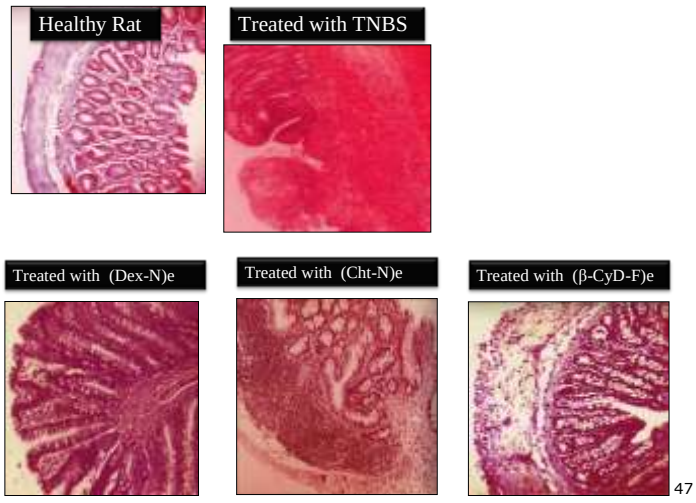


Figure 2: Histological appearance of colonic tissues

The time required and percentage of drug reached the colon was assessed by performing the organ distribution study. Albino rats selected for the organ distribution study were kept in well- spaced ventilated cages and maintained on healthy and fixed diet. These animals were divided into 12 groups, each comprising of 4 rats. Animals of each of the groups were given (β-CyD- Pds),(Dex-Pds),(Cht- Pds) containing equivalent amount of drugs i.e. prednisolone(1mg/kg body weight) orally, followed by sufficient volume of drinking water. The prodrug was suspended in 0.5 ml of 1% Na CMC and administered orally. The doses were given once daily for five consecutive days. Food and water were allowed throughout the period of treatment. After 2, 4, 6 and 8 hour, the rats were humanly killed and stomach, small intestine, colon were isolated. The isolated organs were homogenized with a small volume of PBS (pH 7.4); and 1 mL of acetonitrile was added to homogenate and kept for 30 min. The contents were centrifuged the drug content in the supernatant was determined by UV Spectroscopy.at 240 nm. The drug content in different parts of

the GI tract at different time intervals from 2 hour to 8 hour after drug administration was calculated. The results are given in table 1 and shown in figure 1

Table 1: Organ Distribution Study of Conjugates (% Drugs distributed at different time intervals

Time	Stomach		Small intestine				Colon			
	2hr	4 hr	2 hr	4 hr	6 hr	8 hr	2 hr	4 hr	6 hr	8 hr
(β-CyD- Pds)e	8.68	5.62	0	11.52	13.28	9.81	0	0	41.26	50.12
(Dex- Pds)e	7.52	5.84	0	12.38	13.17	8.82	0	0	34.52	37.58
(Cht- Pds)e	8.48	6.04	0	8.62	12.89	7.22	0	0	31.63	36.68
Prednisolone	50.12	41.26	13.28	12.38	7.22	6.04	3.03	0	0	0

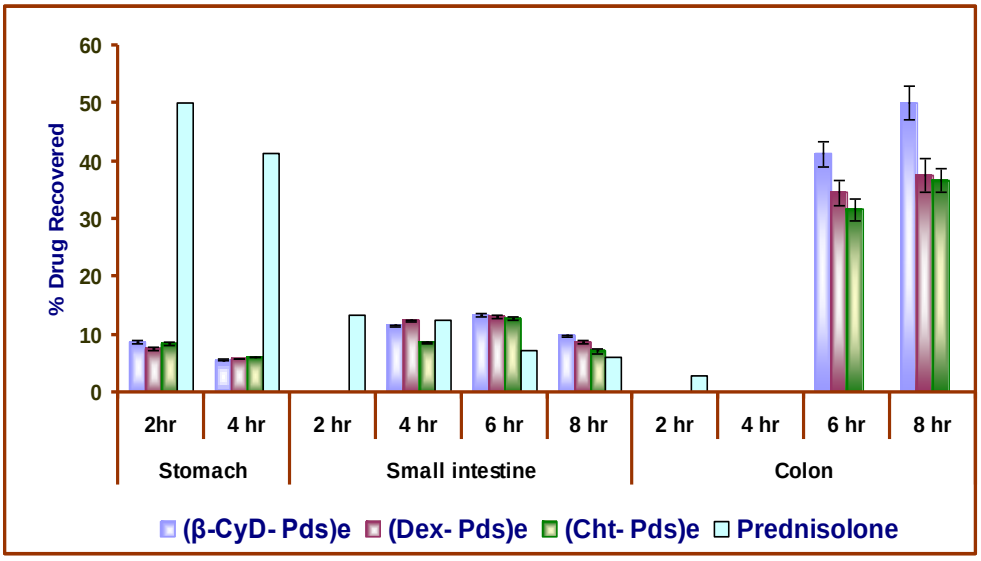


Figure 1: Organ Distribution Study of Conjugates (% Drugs distributed at different time intervals

The recovery of prednisolone from GI tract at various times following oral administration of conjugates suspension is shown in Table 1. During the whole observation period (0-8 h), no Prednisolone conjugates was detected in blood. This observation indicated that conjugates were so stable they could not be degraded in upper GI tract and could not be absorbed into blood. . After 6h,a large portion of the prodrug reached the cecum and colon intact. And unmodified Prednisolone was administered showed that prednisolone was absorbed primarily from the small intestine and the blood concentration of prednisolone was much higher than test groups. Meanwhile, very small amount of prednisolone was observed either in the cecum or in the colon. Finally, The bacterial count in the colon is much higher than that in upper GI tract[2]. The colonic micro flora produce a variety of enzymes, including azoreductase, various glycosidases and amidases, which are not present in the stomach or the small intestine. Therefore, enzyme

dependent drug release, which relies on the existence of enzyme-producing microorganisms in the colon, could be used to deliver drug to the colon after enzymatic cleavage of degradable carrier bonds and premature drug release does not occur in this case. Besides treating inflammatory bowel diseases, colon specific drug delivery system might be useful in other situations. The delivery of certain antineoplastic agents to the. colon might be beneficial in controlling colon cancer[25].Enzyme prodrug gene therapy for colon cancer is also investigated by several researchers[9]. Antibiotics might be delivered specifically to the colon via cyclodextrin carriers[28] In each of these cases, colon-specific delivery would allow the use of higher doses of potent agents with fewer systemic side effects. The present results showed that the ester type prodrugs of Prednisolone. Prednisolone conjugates release Prednisolone. preferentially on caecal and colonic contents in to small oligosaccharides, suggesting that dextran, βcyclodextrin/and chitosan could serve as a new class of colon-specific drug carrier. The dextran , β cyclodextrin and chitosan conjugate survives the passage through upper GI tract although the high level of esterase in small intestine, indicating that dextran,βcyclodextrin and chitosan protects ester bond from hydrolysis by esterase. This result, together with the observation mentioned above, suggests that bacterial enzymes in the colon are responsible for hydrolysis of dextran, βcyclodextrin and chitosan conjugates. When .conjugates reached the colon, dextran, βcyclodextrin and chitosan was completely hydrolyzed into smaller oligosaccharides and exposed the ester bonds to esterase, which led to the rapid release of .Prednisolone.

CONCLUSION

A colon-specific drug-delivery system has been developed based on drug- polysaccharide. conjugation and the unique esterase activity of the colonic microflora. Colonic drug delivery can be achieved with carriers by making prodrugs that survive the passage through stomach and small intestine, but the active moiety is released by enzymes specifically produced in colon.

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