Available online at www.sciencedirect.com
SCIENCE DIRECT
European Journal of Pharmaceutics and Biopharmaceutics 59 (2005) 155–160
European Journal of Pharmaceutics and Biopharmaceutics
www.elsevier.com/locate/ejpb

Research paper

Synthesis and properties of polysaccharide prodrugs of 5-aminosalicylic acid as potential colon-specific delivery systems

Meijuan Zou[a,b], Hirokazu Okamoto[b], Gang Cheng[a], Xiuhua Hao[a], Jin Sun[a], Fude Cui[a], Kazumi Danjo[b,*]

[a]*School of Pharmacy, Shenyang Pharmaceutical University, Shenyang, China*
[b]*Faculty of Pharmacy, Meijo University, Nagoya, Japan*

Received 17 May 2004; accepted in revised form 21 June 2004
Available online 8 September 2004

Abstract

The drug release of the polymer prodrugs of 5-aminosalicylic acid (5-ASA) was not only dependent on the property of the polymers but also dependent on the solubility of the prodrugs. We prepared several polysaccharide prodrugs of 5-ASA to examine the effect of solubility of prodrugs on the release characteristics of 5-ASA in the gastrointestinal contents of rats. The amide prodrug, chitosan-5-ASA (ChT-5-ASA), did not release the 5-ASA in the cecal and colonic contents. The ester prodrugs, hydroxypropyl cellulose-5-ASA (HPC-5-ASA), being poor solubility in 0.05 mol/l acetic acid solution also did not release the 5-ASA in any of gastrointestinal contents of rats. Whereas the 5-ASA release from cyclodextrins-5-ASA (CyDs-5-ASA) in cecal and colonic contents was significantly higher than that in stomach and small intestine contents. And furthermore, with the decrease in the degree of substitution, the solubility of CyD-5-ASA increased, and the release of 5-ASA in the gastrointestinal contents was also higher at the same time interval of incubation. When the ratio of cyclodextrin (CyD) and 5-formylaminosalicylic acid (5-fASA), a precursor of 5-ASA prodrugs, was 1:10, CyD-5-ASA was very slightly soluble, and no release of 5-ASA was observed within 48 h in gastrointestinal contents. The present results suggested that the ester prodrugs of 5-ASA with certain solubility could release 5-ASA in the cecal and colonic contents of rat.

Keywords: Polysaccharide prodrug; Cyclodextrin; Hydroxypropyl cellulose; Chitosan; 5-Aminosalicylic acid; Incubation; Colon-specific

1. Introduction

Oral colon-specific delivery system is naturally of value for the topical treatment of diseases of colon such as ulcerative colitis, Crohn's disease, or colorectal cancer [1–3], whereby high local concentration can be achieved while minimizing side effects. 5-Aminosalicylic acid (5-ASA) is an active ingredient of agents used for the long-term maintenance therapy to prevent relapses of Crohn's disease and ulcerative colitis [4,5]. However, when 5-ASA is administrated orally, a large amount of the drug is absorbed from the upper gastrointestinal tract, and causes systemic side effects.

Polymeric prodrugs of 5-ASA have not been used in clinic. Natural polysaccharides have been used as tools to deliver the drugs especially to the colon. These polysaccharides remain intact in the physiological environment of stomach and small intestine but once the dosage form enters the colon, it is acted upon by polysaccharidases, which releases the drug into colon [6–8].

Because the dose of 5-ASA a day is 1500 mg, the degree of substitution (DS) of the prodrugs should be as much as possible. DS would affect the solubility and drug release characteristics of prodrugs. The CyD prodrugs [9,10] prepared were effective to deliver biphenylylacetic acid to the large intestine in rats; however, the effect of DS was not mentioned. The effect of DS on the release of 5-ASA from the CyDs-5-ASA prodrugs were investigated. 5-ASA is

* Corresponding author. Faculty of Pharmacy, Meijo University, 150 Yagotoyama, Tempaku-ku, Nagoya 468-8503, Japan. Tel.: +81-52-832-1781x273; fax: +81-52-834-8090.
E-mail address: danjo@ccmfs.meijo-u.ac.jp (K. Danjo).

0939-6411/$ - see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejpb.2004.06.004

slightly soluble in water. CyDs-5-ASA with an increased solubility would be suitable for colon-specific delivery system.

CyD [13–15], ChT [12] and HPC were selected as model polymers in the present study. Both ChT-5-ASA and HPC-5-ASA with different molecular weights and DS released negligible amount of 5-ASA in cecal and colonic contents for ChT-5-ASA being amide prodrugs [9,10], and HPC-5-ASA being very poor soluble comparing with dextran-5-ASA of 6 g/l and prednisolone-appended CyD of more than 500 g/l [11]. CyDs-5-ASA can provide a means of colon-specific delivery systems but only with proper DS.

2. Materials and methods

2.1. Materials

HPC was supplied from Nippon Soda Co. Ltd (SSL, 15,000–30,000, Tokyo). α-, β-, γ-CyDs and carbonyldiimidazole (CDI) were obtained from Sigma Chemical Co. (St Louis, USA). 5-ASA was purchased from Acros Organics Ltd (New Jersey, USA), and ChT was supplied from Wako Pure Chemical Industries (deacetylation rate: 80%, 5-20cP, Osaka). DIAION SK1B ion-exchange was obtained from Mitsubishi Chemical Corporation (Tokyo, Japan). All other chemicals and solvents were analytical reagent grade, and deionized double-distilled water was used throughout the study.

2.2. Analytical methods

^{1}H, ^{13}C-NMR spectra were taken on a JNM-GX400 superconducting magnet (JEOL Ltd, Tokyo). Melting and decomposition points were conducted in a DSC-60 100 V AC differential scanning calorimeter (Shimadzu Co., Kyoto). RAD-IIVC X-ray diffraction apparatus performed X-ray diffraction patterns (Rigaku Denki Co. Ltd, Tokyo). The HPLC system consisted of LC-10ADvp pumps, SPD-10ADvp detector and SIL-10ADvp autoinjector from Shimadzu.

Revered-phase HPLC conditions [16,17] for the determination of 5-ASA and its metabolites were as follows: a mobile phase of 5.0 mmol/l pH 6.0 phosphate buffer/acetonitrile/0.1 mol/l tetrabutyl-ammonium chloride (90:10:0.5, v/v), a flow rate of 1.0 ml/min, and a detection of wavelength 330 nm for 0–9 min, 240 nm after 9 min.

2.3. Preparation of CyD (HPC, ChT)-5-ASA ester conjugates

5-ASA (1 g, 6.5 mmol) in 98% formic acid (10 ml) was refluxed for 30 min and 20 ml of cold distilled water was added. The precipitates were filtered, washed several times with cold water, and dried in vacuum. 5-Formylaminosalicylic acid (5-fASA) was obtained with 88% yield, m.p.: 251.19 °C.

To the solution of 5-fASA (1 mmol) in dimethylformamide (DMF 5 ml), CDI (1.5 mmol) was added slowly, and reacted for 1 h at room temperature, and CyD (HPC or ChT 1 mmol) in dimethylsulfoxide (DMSO 10 ml) was added dropwise. To the reaction mixture, triethylamine (TEA 0.8 ml) was added and stirred for 24 h at room temperature, and added excess 1 mol/l HCl or acetone to produce precipitates. CyD (HPC, ChT)-5-ASA was obtained by the hydrolysis of the precipitates in 0.5 mol/l HCl for 10 min at 80 °C.

CyD (HPC, ChT)-5-ASA was purified by DIAION SK1B ion-exchange chromatography.

2.4. Preparation of 5-N-acetyl aminosalicylic acid (N-Ac-ASA)

The *N*-acetyl derivative (*N*-Ac-ASA) was prepared in 41% yield from 5-ASA, acetic anhydride and sulfuric acid treatment. The product exhibited the following: m.p.: 218.13 °C; ^{1}H NMR (dimethyl sulfoxide-d_6) δ 6.8–8.1(complex m, 3, aromatic), 2.00 (s, 3, acetyl). Anal. ($C_9H_9NO_4$).

2.5. Degree of substitution (DS)

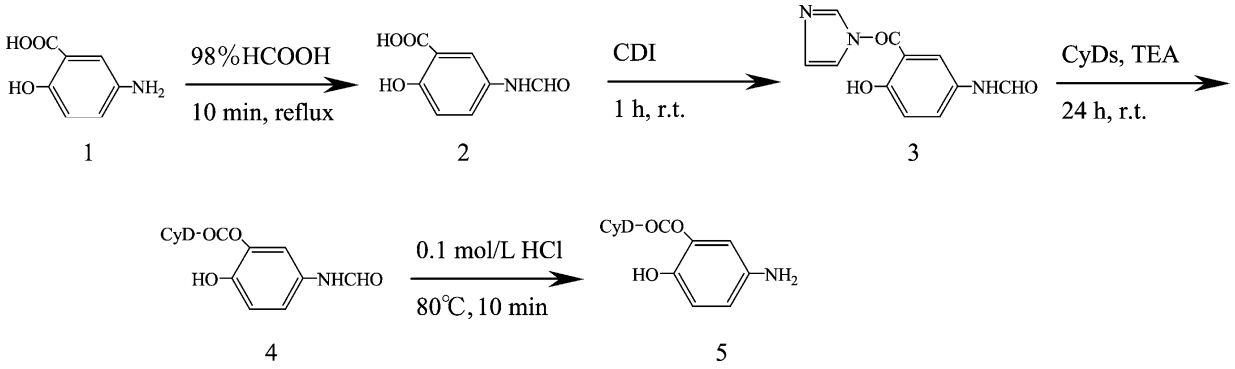
DS was defined as a ratio of the number of hydroxyl groups 5-ASA bound among all hydroxyl groups of cyclodextrins. It was determined by measuring the amount of sodium 5-aminosalicylate by HPLC at 330 nm, which was released when 100 mg of HPC-5-ASA was placed in 1 mol/l NaOH solution for 1 h.

2.6. Chemical stability

CyD-5-ASA (1 g) was placed in solution of 50 ml of pH 1.2, 6.8 or 7.5 buffer and reacted for 6 h at 37 °C. Reaction mixture 0.1 ml and mobile phase 0.9 ml were vortexed for 2 min, centrifuged at 10,000 r/min for 5 min, and the amount of 5-ASA in the supernatant was analyzed by HPLC.

2.7. Measurement of solubility

Screw-capped test tubes containing conjugates, in excess amount, in 2.0 ml of 0.05 mol/l acetic acid solution were stirred for 12 h at 25 °C. The solutions were centrifuged at 3000 rpm for 5 min, and the supernatant was filtered through a membrane filter (Dismic-13cp, 0.20 μm, Toyo Roshi Kaisha, Ltd, Tokyo). One milliliter of the filtrate was placed in 1 ml of 2 mol/l NaOH solution for 1 h. The amount of 5-ASA in the solution was analyzed by HPLC.



Scheme 1. Synthesis of CyD-5-ASA.

2.8. Release of 5-ASA after incubation with the contents of stomach and small intestine of rats

A male SD rat (about 250 g) was anesthetized by diethyl ether and midline incision was made. Contents of stomach and small intestine were collected separately, and the contents were diluted to half concentration with isotonic acetate buffer (pH 4.5) for stomach contents and with isotonic phosphate buffer (pH 6.8) for small intestine contents. To 0.2 g portion of each dilution, 0.8 ml of CyD (HPC, ChT)-5-ASA solution (140 μg equivalent of 5-ASA) was added and the mixture was incubated at 37 °C. At appropriate time intervals, the sample was centrifuged at 5000 rpm for 5 min. To 0.1 ml portion of the supernatant, 1.9 ml of methanol was added, vortexed for 1 min, and filtered through a membrane filter (Dismic-13cp, 0.20 μm, Toyo Roshi Kaisha, Ltd, Tokyo). The concentration of 5-ASA was determinated by HPLC as described previously.

2.9. Release of 5-ASA after incubation with the contents of cecum and colon of rats

The contents of cecum and colon were collected separately in a bag, which was previously displaced by nitrogen. To the solution of CyD (HPC, ChT)-5-ASA (140 μg equivalent of 5-ASA) in pH 7.5 buffer 0.9 ml, which was previously displaced by nitrogen, 0.1 g portion of the gut contents was added and the mixture was incubated at 37 °C. At appropriate time intervals, the sample was centrifuged at 5000 rpm for 5 min. To 0.1 ml portion of the supernatant, 1.9 ml of methanol was added, vortexed for 1 min, and filtered through the membrane filter. The concentration of 5-ASA was determinated by HPLC.

3. Results and discussion

3.1. Preparation of CyD (HPC, ChT)-5-ASA ester conjugates

Preparation of CyD (HPC, ChT)-5-ASA was achieved as shown in Scheme 1. Amino group of 5-ASA was protected by formylation, which proceeded easily in formic acid in good yield. Imidazolide of 5-fASA (**3**) was prepared with excess of CDI, which reacted with CyDs in the presence of TEA as catalyst to form the prodrug (**5**).

Adjusting the ratio of CyD and 5-fASA varied the DS and the results are listed in Table 1. The X-ray diffraction patterns are displayed in Fig. 1. It was shown that the properties of conjugates were different from the 5-ASA and CyDs. From the IR spectra of CyD-5-ASA conjugates, the ester carbonyl peak was observed at 1690 cm^{-1} in addition to the peaks originated from 5-ASA and β-CyDs (Fig. 2). ^{1}H NMR of CyD-5-ASA was δ 3.6–6 (CyDs), δ 6.6–8.1 (5-ASA). ^{13}H NMR of CyD-5-ASA was that the portion of CyDs was δ 98–101.4 (1-C), δ 81.1 (4-C), δ 69–72 (3,5,2-C), and δ 59.78 (6-C) and the portion of 5-ASA was δ 162–169 (COOH), δ 152–158 (2-C–OH), δ 139 (5-C–NH), δ 127–130 (6-C), δ 117–123 (3,4-C), and δ 111–113 (1C).

3.2. Chemical stability

It was noted that no free 5-ASA was detected regardless of the value of DS, which suggested that all the prodrugs

Table 1
The DS of the resulting prodrugs

CyDs:5-fASA (mole ratio)	DS (5-fASA) (%)			DS (5-ASA) (%)		
	α-CyD	β-CyD	γ-CyD	α-CyD	β-CyD	γ-CyD
1:1	6.7	6.2	6.7	6.1	5.7	6.3
1:2	15.6	12.9	13.3	14.4	12.4	11.7
1:6	38.3	35.2	32.9	37.8	33.3	31.7
1:10	45.0	41.9	37.9	43.9	37.1	37.5

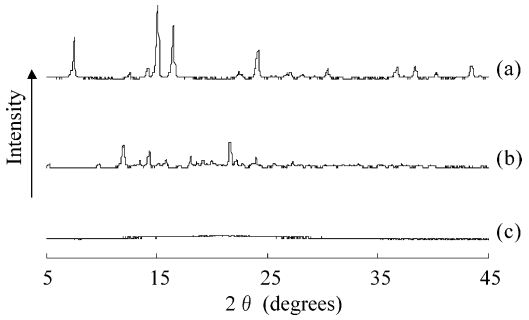


Fig. 1. X-ray diffraction patterns of 5-ASA (a), α-CyD (b) and α-CyD-5-ASA (c).

listed in Table 1 were chemically stable at pHs 1.2, 6.8, and 7.5.

3.3. Aqueous solubility

Table 2 summarizes some physiochemical properties of the conjugates. It was apparent that the decomposition point of the conjugates decreased with the increase in DS, and the solubility decreased even lower than that of 5-ASA. The solubility of CyDs-5-ASA with DS of about 6.7 and 15.6 increased greatly in comparison to that of free 5-ASA. CyDs-5-ASA with DS more than 30.0 was very slightly soluble, which maybe the hindering factor for the release of 5-ASA. The solubility of HPC-5-ASA and ChT-5-ASA was lower than that of 5-ASA.

3.4. Release of 5-ASA after incubation with the contents of gastrointestinal tract of rat

All the prodrugs used for the incubation study was the ratio 1:2 of cyclodextrin (CyD) and 5-formylaminosalicylic acid (5-fASA) and the results are shown in Fig. 3. It was indicated that HPC-5-ASA and ChT-5-ASA did not release the 5-ASA in any of the contents of rats. In the contents of stomach and small intestine, the CyDs-5-ASA released 5-ASA only in small amounts (<12%). In the cecal and colonic contents CyDs-5-ASA released 5-ASA significantly greatly. On the other hand, the release of 5-ASA was in the order of γ-CyD-5-ASA, α-CyD-5-ASA, and β-CyD-5-ASA because the solubility of CyDs was in the order of γ-CyD, α-CyD, and β-CyD, β-CyD with the lowest solubility. The release of 5-ASA from α-CyD-5-ASA and γ-CyD-5-ASA having no significant difference may be a steric effect of the bulky CyD moiety.

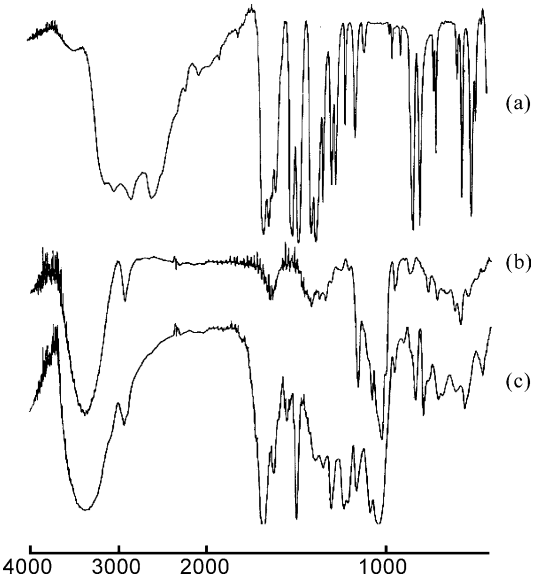


Fig. 2. IR spectra of 5-ASA (a), β-CyD (b) and β-CyD-5-ASA (c).

Table 2
Some physiochemical properties of 5-ASA and resulting conjugate

Compound	CyDs:5-fASA (mole ratio)	FW	Decomposition or melting point (°C)	Solubility (g/l)
5-ASA	–	153.1	255.2 (m.p.)	1.0
HPC-5-ASA	–	–	–	<0.05
ChT-5-ASA	–	–	–	<0.05
α-CyD		972.9	277.14 (m.p.)	145.0
β-CyD		1135.0	283.72 (m.p.)	18.5
γ-CyD		1297.2	290.96 (m.p.)	232.0
α-CyD-5-ASA	1:1	1121.4	285.03	396.6
	1:2	1323.9	281.35	174.4
	1:6	1890.9	272.71	2.8
	1:10	2039.4	264.37	0.2
β-CyD-5-ASA	1:1	1297.0	288.27	202.8
	1:2	1675.2	269.38	91.8
	1:6	2080.0	268.18	0.8
	1:10	2188.0	265.90	0.03
γ-CyD-5-ASA	1:1	1350.9	286.54	720.5
	1:2	1675.2	271.03	376.2
	1:6	2323.2	260.37	16.3
	1:10	2512.2	252.02	0.2

The effect of DS on the release of 5-ASA from α-CyD-5-ASA was investigated by the incubation of α-CyD-5-ASA with different DS in rat colonic contents and the results were shown in Fig. 4. The release of 5-ASA increased with the decrease in DS of α-CyD-5-ASA. The solubility of α-CyD-5-ASA with DS of 6.7, 15.6, 38.3 and 45.0 was 396.6, 174.4, 2.8 and 0.2 g/l and the degree of 5-ASA released was 100.0, 81.9, 7.1 and 0.0% in 12 h, respectively. The result indicated that a DS of prodrug higher than 30 may be not suitable as a colon-specific prodrug of 5-ASA.

Furthermore the effect of different type of HPC (SSL, SL, L, M, H) or DS (7.8, 16.3) on the release of 5-ASA from prodrugs was also investigated and no 5-ASA was detected in the cecal and colonic contents of rats. ChT undergoes degradation by the action of colonic microflora, and hence several ChT-5-ASA conjugates were produced, but no release of 5-ASA was observed in any of gastrointestinal tract contents of rats for its being ester bond between the 5-ASA and ChT.

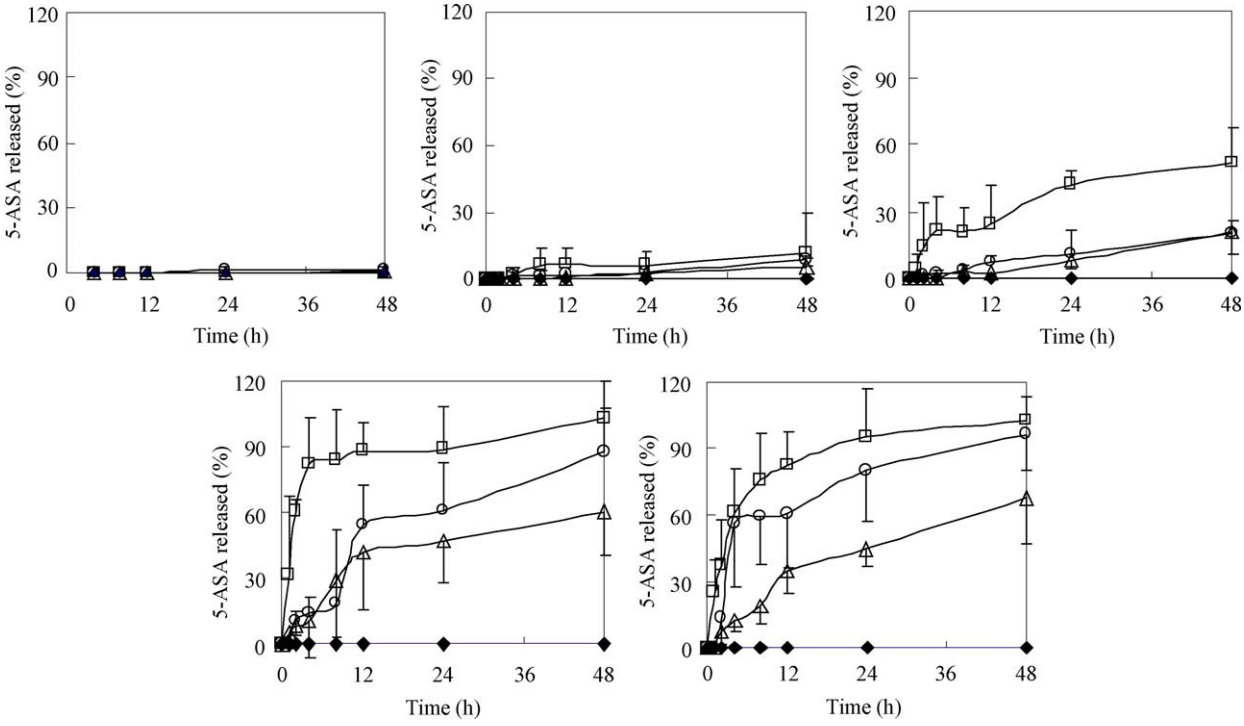


Fig. 3. Time courses of 5-ASA liberation from ChT-5-ASA, HPC-5-ASA (♦), α-CyD-5-ASA (○), β-CyD-5-ASA (△) and γ-CyD-5-ASA (□) in rat intestinal contents and biological fluids in isotonic buffer solution after incubation at 37 °C:panel A, pH 7.4 buffer; panel B, stomach contents; panel C, small intestinal contents; panel D, cecal contents; panel E, colonic contents $n=6$.

4. Conclusions

Formation of prodrugs has improved delivery properties over the parent drug molecule. In this study, three pharmacologically inactive polysaccharides, HPC, ChT and CyD were used as carriers. The results showed that no release of 5-ASA from HPC-5-ASA and ChT-5-ASA was detected in any of the contents of rats. And 5-ASA liberated from CyDs-5-ASA was in small amount after incubation with the contents of stomach and small intestine of rats. On the basis of the incubation data, the release of 5-ASA from the CyDs-5-ASA in the cecum and colon was relative to the DS or the solubility of CyD-5-ASA. The solubility of α-CyD-5-ASA with DS of 6.7, 15.6, 38.3 and 45.0 was 396.6, 174.4, 2.8 and 0.2 g/l, respectively. Comparing with the colon-specific delivery prodrugs dextran-5-ASA and prednisolone-appended CyD with the solubility of more than 6 and 500 g/l, the solubility of α-CyD-5-ASA with DS 45.0 was so low that it cannot release the 5-ASA in colonic content even in 48 h. Therefore, the CyD prodrugs approaches with DS lower than 30 can perhaps provide a delayed-release colon-specific delivery system.

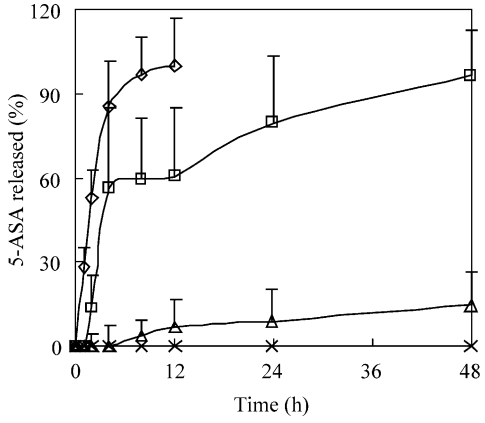


Fig. 4. Time courses of 5-ASA liberation from α-CyD-5-ASA with DS=6.7 (◇), DS=15.6 (□), DS=38.3 (△) and DS=45.0 (×) in rat colonic contents after incubation at 37 °C $n=6$.

References

[1] E. Schacht, A. Gevaert, E.R. Kenawy, K. Molly, W. Verstraete, P. Adriaensens, R. Caleer, J. Gelan, Polymers for colon specific drug delivery, J. Control. Release 39 (1996) 327–338.
[2] A. Gazzaniga, P. Iamartino, G. Maffione, M.E. Sangall, Oral delayed-release system for colonic specific delivery, Int. J. Pharm. 108 (1994) 77–84.
[3] J.P. Brown, G.V. McGarraugh, T.M. Parkinson, R.E. Wingard, A.B. Onderdonk, A polymeric drug for treatment of inflammatory bowel disease, J. Med. Chem. 26 (1983) 1300–1307.
[4] E. Carceller, J. Salas, M. Merlos, M. Giral, R. Ferrando, I. Escamilla, J. Ramis, J. Garcia-Rafanell, J. Forn, Novel azo derivatives as prodrugs of 5-aminosalicylic acid and amino derivatives with potent platelet activating factor antagonist activity, J. Med. Chem. 44 (2001) 3001–3013.

[5] Y.J. Jung, J.S. Lee, Y.M. Kim, Colon-specific prodrugs of 5-aminosalicylic acid: synthesis and in vitro/in vivo properties of acidic amino acid derivatives of 5-aminosalicylic acid, J. Pharm. Sci. 90 (2001) 1767–1775.
[6] V.R. Sinha, R. Kumria, Polysaccharides in colon-specific drug delivery, Int. J. Pharm. 224 (2001) 19–38.
[7] P.J. Watts, L. Illu, The colonic delivery system, Drug. Dev. Ind. Pharm. 23 (1997) 893–913.
[8] M.K. Chourasia, S.K. Jain, Pharmaceutical approaches to colon targeted drug delivery systems, J. Pharm. Pharm. Sci. 6 (2003) 33–66.
[9] K. Minami, F. Hirayama, K. Uekama, Colon-specific drug delivery based on a cyclodextrin prodrug: release behavior of biphenylylacetic acid from its cyclodextrin conjugates in rat intestinal tracts after oral administration, J. Pharm. Sci. 87 (1998) 715–720.
[10] K. Uekama, K. Minami, F. Hirayama, 6A-*O*-[(4-biphenylyl)acetyl]-α-, β- and γ-cyclodextrins and 6A-deoxy-6A-[[(4-biphenylyl)acetyl] amino]-α-, β- and γ-cyclodextrins: potential prodrugs for colon-specific delivery, J. Med. Chem. 40 (1997) 2755–2761.
[11] H. Yano, F. Hirayama, M. Kamada, H. Arima, K. Uekama, Colon-specific delivery of prednisolone-appended α-cyclodextrin conjugate: alleviation of systemic side effect after oral administration, J. Control. Release 79 (2002) 103–112.
[12] H. Yano, F. Hirayama, H. Arima, K. Uekama, Prednisolone-appended α-cyclodextrin: alleviation of systemic adverse effect of prednisolone after intracolonic administration in 2,4,6-trinitrobenzenesulfonic acid-induced colitis rats, J. Pharm. Sci. 90 (2002) 2103–2112.
[13] H. Yano, F. Hirayama, H. Arima, K. Uekama, Preparation of prednisolone-appended α-, β- and γ-cyclodextrins: substitution at secondary hydroxyl groups and in vitro hydrolysis behavior, J. Pharm. Sci. 90 (2001) 493–503.
[14] H. Tozaki, T. Fujita, T. Odoriba, A. Terabe, S. Okabe, S. Muranishi, A. Yamamoto, Validation of a pharmacokinetic model of colon-specific drug delivery and the therapeutic effects of chitosan capsules containing 5-aminosalicylic acid on 2,4,6-trinitrobenzenesulphonic acid-induced colitis in rats, J. Pharm. Pharmacol. 51 (1999) 1107–1112.
[15] Y.J. Jung, J.S. Lee, H.H. Kim, Y.T. Kim, Y.M. Kim, Synthesis and properties of dextran-5-aminosalicylic acid ester as a potential colon-specific prodrug of 5-aminosalicylic acid, Arch. Pharm. Res. 21 (1998) 179–186.
[16] V.S. Chungi, G.S. Rekhi, L. Shargel, A simple and rapid liquid chromatographic method for the determination of major metabolites of sulfasalazine in biological fluids, J. Pharm. Sci. 78 (1989) 235–238.
[17] F.N. Hussain, R.A. Ajjan, M. Moustafa, J.C. Anderson, S.A. Riley, Simple method for the determination of 5-aminosalicylic and *N*-acetyl-5-aminosalicylic acid in rectal tissue biopsies, J. Chromatogr. B. Biomed. Sci. Appl. 716 (1998) 257–266.