Degradability of the Linear Azo Polymer Conjugated 5,5′-Azodisalicylic Acid Segment in the Main Chain for Colon-Specific Drug Delivery

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ABSTRACT: A novel type of linear copolymer composed of poly(ethylene glycol) (PEG) with 5, 5′-azodisalicylic acid [olsalazine (OLZ)] was developed for colon-specific drug delivery. These copolymers contained azo bonds that would be degraded by the azoreductase activities in the colon. The resultant condensation polymers were characterized with Fourier transform infrared, nuclear magnetic resonance, and gel permeation chromatography. The degradation behavior of the polymer was evaluated *in vitro* and *in vivo*. The *in vitro* results indicated that the active 5-aminosalicylic acid (5-ASA), one of the degradation products, could be released in the medium of the cecum contents specifically. In an *in vivo* test, there was a 8-h lag time before 5-ASA could be detected in urine samples, and this indicated that the conjugate could remain intact in the upper part of the gastrointestinal tract. In comparison with OLZ, the release profiles of 5-ASA from PEG–OLZ copolymers were significantly prolonged. In addition, the release profiles of 5-ASA from PEG–OLZ copolymers could be adjusted by changes in the molecular weight of the PEG segment. Because of these advantages of PEG–OLZ copolymers, it could be concluded that PEG–OLZ copolymers could be promising candidates for colon-specific polymeric prodrugs of 5-ASA. © 2008 Wiley Periodicals, Inc. J Appl Polym Sci 108: 3305–3312, 2008

Key words: azo polymers; degradation; drug delivery systems; 5-aminosalicylic acid; poly(ethylene glycol)

INTRODUCTION

Oral drug delivery is preferred by a majority of patients because it is a convenient and patient-friendly route of drug administration in comparison with injections. In most cases, oral drugs are released in the upper gastrointestinal tract. However, sometimes it may be beneficial to release them in the colon to, for example, minimize side effects and to maximize the therapeutic response. Recently, various approaches have been used to deliver the drug molecules to the colon site specifically,[1–3] such as the use of polymeric coatings and matrices and the application of bonded drugs containing inert carriers and active agents (i.e., prodrugs). Site-specific delivery to the colon can be achieved by the exploitation of the specific microbial enzyme activities (e.g., azoreductase) present in the colon. The azoreductase activities in the colonic fluid can be used to convert low-molecular-weight prodrugs into active metabolites[4–7] and to release active species from water-soluble polymeric carriers.[8–14]

Recently, we studied the synthesis and properties of a novel type of linear azo polymer for colon-specific drug delivery.[15] The azo copolymers consisted of covalently bonded olsalazine (OLZ) moieties on the hydrophilic and biocompatible poly(ethylene glycol) (PEG) segments. OLZ has been successfully used in clinics as an orally administrated drug. This drug converts to the therapeutically active mesalamine [5-aminosalicylic acid (5-ASA)] component by colonic bacteria. PEG was selected as a drug carrier polymer because of its excellent biocompatibility and safety. By the variation of the chain length of the PEG segments (number-average molecular weight = 400, 1000, 4000, or 10,000), the physical and physiological properties of PEG–OLZ copolymers, such as the hydrophilicity and biodegradability, could be modulated. The cleavage rate of the ester bonds and azo bonds was evaluated by hydrolysis and biodegradation with Sprague–Dawley (S–D) rat cecum contents *in vitro*. It is believed that with the azo bond split by bacterial azoreductase and the ester bond split by hydrolysis in the colon, the active moiety 5-ASA can be released, and this should prevent the adverse effects.

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In this work, PEG–OLZ copolymers were synthesized, and the degradability of the PEG–OLZ copolymers was determined with rat gastrointestinal contents *in vitro* and by oral administration to S–D male rats *in vivo*. Released 5-ASA was quantitatively analyzed with a high-performance liquid chromatography (HPLC) assay.

EXPERIMENTAL

Materials

5,5′-Azodisalicylic acid was purchased from Jinhua Zhongyi Pharmaceutical Co. (Jinhua, China). PEG (number-average molecular weight = 400, 1000, 4000, or 10,000), succinic anhydride, α-D-glucose, 5-ASA, *N*-acetyl-5-aminosalicylic acid (ace-5-ASA), dicyclohexyl carbodiimide (DCC), and *N,N*′-dimethyl amino pyridine (DMAP) were purchased from Acros (Geel, Belgium). Benzyl viologen (BV) was obtained from Aldrich (St. Louis, MO); sodium heparin was purchased from Shanghai Bio-Life Science and Technology Co., Ltd. (Shanghai, China). PEG and ethylene glycol were dried at 70°C *in vacuo* for 12 h before use. Succinic anhydride was purified by recrystallization from acetic anhydride before use. DCC was distilled under low pressure (bp = 130°C at 3 mmHg). Tetrahydrofuran (THF) was dried with CaH_2 and distilled. The other chemicals were used as received.

Characterization

Infrared (IR) spectra were recorded with a Bruker Vector 22 Fourier transform infrared instrument (Billerica, MA). ^{1}H- and ^{13}C-NMR spectra were obtained with an Avance DMX 500-MHz spectrometer (Billerica, MA) using dimethyl sulfoxide-d_6 (DMSO-d_6) as a solvent. The molecular weight of the azo polymers was measured with a Waters model 208 gel permeation chromatograph (Milford, MA) with THF as the mobile phase as well as the sample solvent. The degradation of PEG–OLZ copolymers *in vitro* was monitored by ultraviolet–visible (UV–vis) spectroscopy on a Cary 100 Bio UV–vis spectrophotometer (Palo Alto, CA). HPLC was employed to detect the biodegraded products of the azo copolymers *in vivo*. The analysis was performed on an HPLC system (Shimadzu, Kyoto, Japan), which was equipped with a binary bump (LC_10ADVP), a reversed-phase column (Diamosil C_{18}, 5-μm pore size, 250 × 4.6 mm), and a UV–vis absorption detector (SPD-10AVP).

Synthesis of di(2-hydroxyethyl)-5,5′-azodisalicylate (I)

I was synthesized by esterification with DCC as the coupling agent and DMAP as the basic catalyst.[16–19] OLZ (0.60 g, 2 mmol) and ethylene glycol (0.40 g, in excess) were dissolved in 40 mL of THF in a sealed flask at room temperature. After nitrogen purging for 30 min, DMAP (0.07 g, 0.6 mmol) and DCC (1.03 g, 5.0 mmol) in 10 mL of THF were added successively. The reaction lasted for 24 h at room temperature under nitrogen atmosphere protection with stirring. At the completion of the reaction, the precipitated dicyclohexylurea was removed by filtration. The filtrate was precipitated into toluene. The product was collected by filtration and dried *in vacuo* (yield = 73%). The dried product was characterized with IR, ^{1}H-NMR, and ^{13}C-NMR.

IR (KBr): 1675 (s, C=O), 1560 (vs, C=C of phenyl), 2925 cm^{-1} (s, —O—CH_2CH_2O—). ^{1}H-NMR (DMSO-d_6, δ): 3.77 (t, 4H, —CH_2—OH), 4.39 (t, 4H, —CH_2—CH$_2$—OH), 7.17 (m, 2H, meta of —COO—), 8.05 (m, 2H, ortho of —COO—), 8.36 (t, 2H, para of —COO—), 10.95 (s, 2H, —O*H*), 5.25 (s, 2H, CH$_2$—O*H*). ^{13}C-NMR (DMSO-d_6, δ): 59.3 (COO—*C*H$_2$), 67.8 (CH$_2$*C*H$_2$—OH), 114.4 (5-C of phenyl), 119.1 (1-C of phenyl), 126.6 (2-C of phenyl), 128.5 (4-C of phenyl), 144.9 (3-C of phenyl), 162.6 (6-C of phenyl), 168.6 (*C*OOCH$_2$).

Synthesis of dicarboxylated poly(ethylene glycol) (II)

II (number-average molecular weight = 400, 1000, 4000, or 10,000) was prepared with the procedures described by Zalipsky et al.[20] and Bea et al.[21] Briefly, dried PEG (weight-average molecular weight = 1000; 10.00 g, 10 mmol) was dissolved in 1,4-dioxane (50 mL). Succinic anhydride (2.50 g, 25 mmol), DMAP (2.44 g, 20 mmol), and triethylamine (20 mmol) were added to the solution and stirred for 24 h at room temperature under anhydrous conditions. After the reaction was finished, the dioxane was evaporated *in vacuo*. The residue was dissolved in CCl_4, and the solid was filtered off. The filtrate was precipitated into diethyl ether, filtered, and dried *in vacuo* (yield = 88%). The derivatization of PEG with molecular weights of 400, 4000, and 10,000 was performed with the same procedure. The dried product was characterized with IR, ^{1}H-NMR, and ^{13}C-NMR.

IR (KBr): 1735 (vs, C=O), 1645 (vs, C=N$^+$), 1559 (vs, COO$^-$), 1115 cm^{-1} (vs, CH$_2$CH$_2$OCH$_2$CH$_2$). ^{1}H-NMR ($CDCl_3$, δ): 4.24 (t, 4H, OCH$_2$C*H*$_2$OCO), 3.64–3.7 (m, 8H, C*H*$_2$C*H*$_2$O), 2.62–2.66 (m, 8H, COC*H*$_2$ C*H*$_2$COOH), 7.83 (s, 2H, COO*H*), 3.17 [s, 6H, (C*H*$_3$)$_2$N of DMAP], 6.66 (d, 2H, pyridine-H of DMAP), 8.30 (d, 2H, pyridine-H of DMAP). ^{13}C-NMR ($CDCl_3$, δ): 29.8 (COCH$_2$CH$_2$CO), 39.7 (CH$_3$ of DMAP), 63.7 (CH$_2$CH$_2$O—CO—), 68.9 (*C*H$_2$CH$_2$O—CO—), 70.1 (O—*C*H$_2$CH$_2$—O), 106.4 (pyridine-3C of DMAP), 141.9 (pyridine-2C of DMAP), 156.6

(pyridine-3C of DMAP), 172.7 (COO^-), 175.6 ($OCOCH_2CH_2COO^-$).

Synthesis of the PEG–OLZ multiblock copolymers

Equimolar amounts (1 mmol) of **I** and **II** were dissolved in 25 mL of THF. DMAP (0.037 g, 0.3 mmol) and DCC (0.618 g, 3 mmol) in 5 mL of THF were added and stirred for 3 days at room temperature under the dried nitrogen atmosphere. When the reaction was completed, the precipitated dicyclohexylurea was removed by filtration. The filtrate was precipitated into cold diethyl ether (4°C). The product was collected by filtration and dried *in vacuo* (yield = 85%).

Degradability of the PEG–OLZ copolymers in a stomach/small intestinal/cecum medium *in vitro*

Five healthy S–D male rats (supplied by the Experiment Animal Center, Medical School, Zhejiang University) weighing 200–250 g were used in the *in vitro* study. Cecum contents were isolated from freshly sacrificed S–D male rats, suspended (1.0-g fresh weight/25 mL) in a 0.1*M* potassium phosphate buffer of pH 6.8, and filtered through glass wool under N_2. The suspension was divided into 1-mL fractions and kept frozen at −20°C. In this experiment, as indicated, the suspensions were used after 14 h of preincubation in 0.1*M* phosphate buffer containing α-D-glucose.

With similar manipulation, sections of the stomach and small intestine were collected separately; the stomach contents were diluted with citric acid buffer (pH 2.5), and the small intestinal contents were diluted with phosphate buffer (pH 6.8).

A 10-mL cell suspension (1.0-g fresh weight/25 mL, containing α-D-glucose, 14 h of preincubation) was mixed with 1 mL of 1 m*M* BV and 5 mL of an OLZ or PEG–OLZ copolymer solution (0.32 m*M* of azo bonds) under nitrogen. The final concentrations of the enzyme-containing solution were 0.025 g/mL of cecum/stomach/small intestine, 0.1 m*M* azo substrate, 62.5 μM BV, and 1.25 mg/mL α-D-glucose. The solution was incubated under anaerobic conditions at 37°C in a shaking chamber. At a predetermined time interval, 1 mL of the solution was taken out under nitrogen and diluted to 1:2 with water before analysis. The release of OLZ segments was monitored with the UV–vis spectrophotometer at 361 nm.

Degradation studies of the PEG–OLZ copolymers in S–D rats *in vivo*

Five groups (five rats per group) of S–D male rats (weighing 225–250 g) were used for evaluating the degradability of PEG–OLZ copolymers during 96 h. A saline solution or suspension (1 mL) containing OLZ or PEG–OLZ copolymer (0.1 mmol equiv of OLZ/kg) was administered via a flexible catheter into the stomach of the rats, which were fasted for 24 h before this study. After administration, the animals were kept in separate cages and fed daily with 20 g of a standard diet in one portion.

Blood samples (0.2 mL) were periodically collected and centrifuged at 1500 rpm for 10 min to obtain plasma samples. Each plasma sample (0.1 mL) was mixed with an equal volume of methanol for 10 s (vortex mixer). The supernatant was separated from the protein pellet by centrifugation (5 min at 12,000 rpm). Urine and feces were collected from experimental rats at time intervals up to 96 h after dosing. One gram of each urine or feces sample was mixed immediately (vortex mixer) with 10 mL of phosphate buffer (0.1 mol/L, pH 6.8). The urine or feces water extract was obtained by subsequent centrifugation (10 min at 4000 rpm). To remove protein, the urine and feces water extract was mixed with a 10-fold volume of methanol, and the supernatant was obtained by centrifugation (5 min at 12,000 rpm).

The quantity of 5-ASA and its metabolites in the biological samples was determined by analysis of the concentration of ace-5-ASA with HPLC, as this material afforded much better spectral linearity than 5-ASA.[9] Acetic anhydride (10 μL) was added to the biological samples, which were shaken for 20 min to convert 5-ASA and metabolites to ace-5-ASA. Thereafter, the solvent was filtered through a membrane filter (0.45 μm) and analyzed with HPLC.

The running conditions of HPLC were similar to those reported by Knoll et al.[7] The mobile phase consisted of a mixture of 0.1*M* acetic acid and 0.4% (v/v) triethylamine (pH 4.3) with acetonitrile in the ratio of 89.5 : 10.5 (v/v). Before analysis, the mobile phase was filtered (0.45 μm) and degassed by means of an ultrasonic bath. The flow rate was 1 mL/min. The injection volume was 20 μL. The elution was monitored by UV absorption at 254 nm.

RESULTS AND DISCUSSION

Synthesis and characterization of the PEG–OLZ multiblock copolymers

The direct copolymerization of OLZ with PEG is difficult to execute and frequently results in low coupling efficiency. We found that conversion to **I** greatly increased the reactivity of OLZ with **II** (Fig. 1). The structure of the azo polymers was confirmed by the ^{1}H- and ^{13}C-NMR analysis (Figs. 2 and 3). In the ^{1}H-NMR spectrum, the peaks at 7.0–8.5 ppm belonged to phenyl, and the peak at 3.7 ppm was characteristic of the main-chain methylene units in

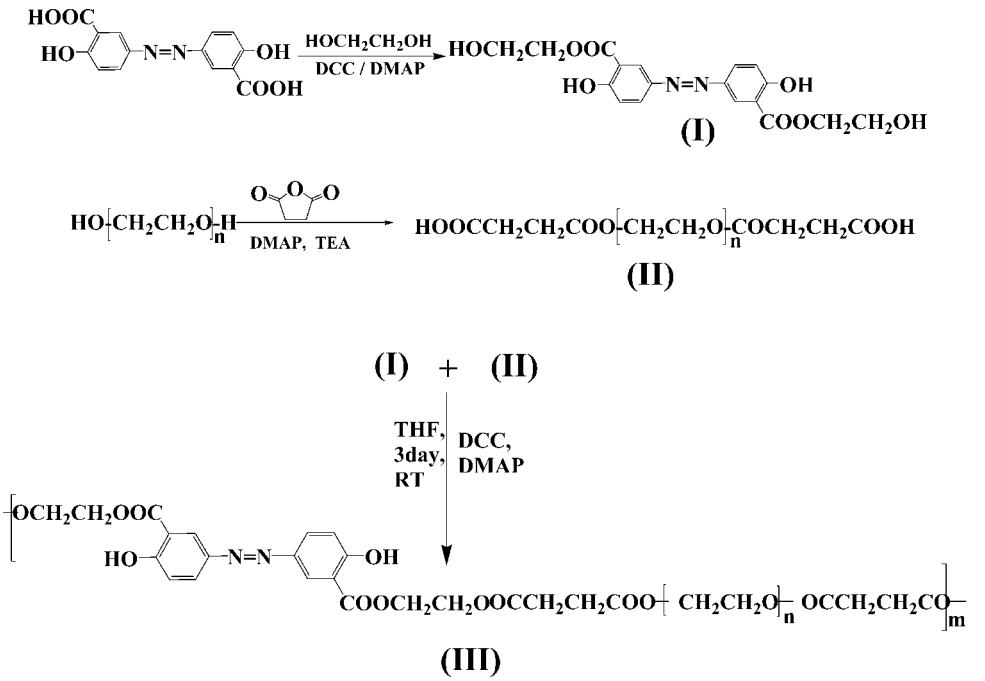

Figure 1 Synthesis of the PEG–OLZ copolymer.

the PEG blocks. The resonance of the phenolic hydroxyl was observed at 10.95 ppm. In the ^{13}C-NMR spectrum, the carbons of benzene appeared at 112–165 ppm, and the peak at 70 ppm belonged to the carbons of the PEG main chain. The peaks at 169–173 ppm were characteristic of carbons of carboxylate.

Despite four hydroxyl groups present in **I**, the carboxy-terminated PEG macromers mainly reacted with the end hydroxyl groups of the ethylene glycol moiety in **I**. This might be the result of higher esterification reactivity for the end hydroxyl group of the ethylene glycol moiety than for the phenolic OH group under ordinary condensation conditions.

The degree of polymerization of the azo polymers depended significantly on the molecular weight of PEG. It is shown in Table I that the degrees of polymerization of the copolymers decreased with the increase in the molecular weight of PEG. The prepared PEG–OLZ copolymers were soluble in common organic solvents such as THF and chloroform.

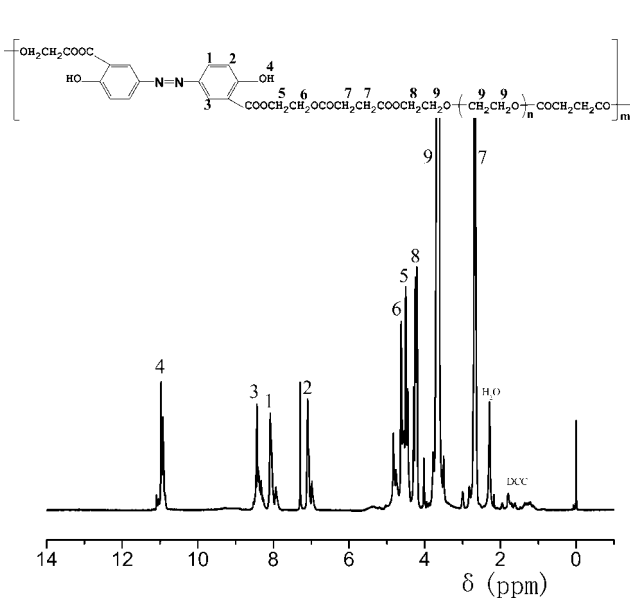

Figure 2 ^{1}H-NMR spectrum of the PEG–OLZ copolymer in $CDCl_3$.

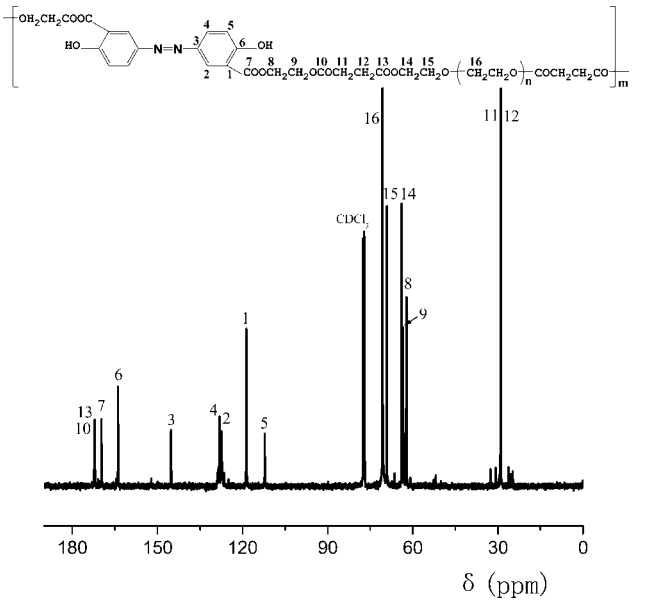

Figure 3 ^{13}C-NMR spectrum of the PEG–OLZ copolymer in $CDCl_3$.

TABLE I
Characterization of the Azo Copolymers

Copolymer	M_n[a]	M_w[b]	PDI[c]	DP[d]	T_m (°C)[e]	Hydration (%)[f]
PEG_{400}–OLZ	5148	7375	1.43	12	<−60	8.9
PEG_{1000}–OLZ	9853	20,499	2.08	9.5	<−60	32.8
PEG_{4000}–OLZ	23,068	33,566	1.46	3.4	23	73
PEG_{10000}–OLZ	46,766	61,841	1.32	4.5	37	87.5

[a] Number-average molecular weight.
[b] Weight-average molecular weight.
[c] Polydispersity index determined by gel permeation chromatography.
[d] Degree of polymerization.
[e] Melting temperature of the copolymer.
[f] $(W_s - W_d)/W_d$, where W_s and W_d are the weights of hydrated and dried samples, respectively.

When the molecular weight of the PEG segment was above 1000, the melting points of the resultant PEG–OLZ copolymers changed toward room temperature.

The hydration behavior of the PEG–OLZ copolymers was determined by incubation of the dried films in 37°C/82% relative humidity desiccators. The hydration degrees of the four azo polyesters were 8.9% for PEG_{400}–OLZ, 32.8% for PEG_{1000}–OLZ, 73% for PEG_{4000}–OLZ, and 87.5% for PEG_{10000}–OLZ (listed in Table I). By the variation of the molecular weight of PEG, the hydration degree of the copolymers could be adjusted readily.

In vitro degradability of the PEG–OLZ copolymers

This experiment was designed to demonstrate that PEG–OLZ copolymers could be degraded and a free drug of 5-ASA could be released efficiently under artificial colon conditions. In our previous cecum bacterial whole-cell experiments,[15] when BV was added as a redox mediator to the cecum incubation medium, the azo bonds in OLZ were reduced up to about 90%, and the azo bonds in the PEG–OLZ copolymers were reduced by more than 50% within 4 h. At the same time, following the azo bond degradation, 5-ASA was released when the ester linkages hydrolyzed. It was found that 5-ASA was released in a sustained fashion from the aqueous PEG–OLZ copolymer solution with cecum contents in a period of more than 32 h. It was also found that the PEG_{4000}–OLZ copolymer exhibited less degradation activity than the PEG_{10000}–OLZ copolymer, and this was attributed mainly to the difference in their hydrophilicity (see the hydration data in Table I). These results imply that the biodegradation of PEG–OLZ copolymers could be readily modulated by changes in the molecular weight of the PEG segments in the PEG–OLZ copolymers.

In this experiment, OLZ and PEG–OLZ copolymers were also incubated with various segments of gastrointestinal tracts of S–D rats at 37°C, and the diminishment of the azo bonds was monitored. Figure 4 illustrates that the diminishment of the azo bonds was more than 50% after 5 h of incubation in rat cecum contents with BV added. In contrast, the azo bond reduction was less than 10% in the small intestinal reductive medium after 5 h of incubation, and very little reduction (%) could be detected in the stomach reductive medium.

In vitro degradation studies performed on different intestinal sections of S–D rats showed that the azoreductase in the rat stomach was scarcely present. Therefore, the azo polymers may transit unchangeably through the rat stomach. The stability of the azo polymers in the small intestine may be attributed to the feeble azo-reducing activity and the short residual time in the small intestine of rats, which has been reported to be less than 5 h.[22] The results indicate that with the azo bond split by bacterial azoreductase and the ester bond split by hydrolysis, PEG–OLZ copolymers can specifically deliver 5-ASA to the colon.

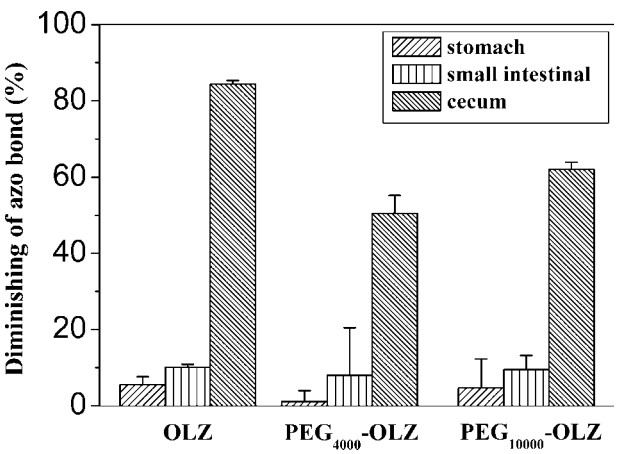

Figure 4 Diminishment of azo bonds in OLZ and PEG–OLZ copolymers in the contents of the stomach, small intestine, and cecum of male S–D rats (whole cell, 14 h of preincubation) at 37°C in the presence of BV and α-D-glucose after 24 h of incubation (azo bond concentration = 0.1 m*M*; BV concentration = 6.25×10^{-5} *M*; cell suspension concentration = 0.025 g/mL; α-D-glucose concentration = 1.25 mg/mL).

In vivo degradability of the PEG–OLZ copolymers

The aim of this study was to elucidate whether PEG–OLZ copolymers were able to pass the intestinal barrier after oral administration and then to realize their degradability. As mentioned previously, PEG–OLZ copolymers under reductive colon conditions demonstrated good degradability *in vitro*. One may predict that PEG–OLZ copolymers might be degradable after oral administration to S–D rats as well. The whole-cell data *in vitro* were employed to estimate the *in vivo* reduction rate of azo bonds in the rats. On the basis of the maximum reduction rate (0.83 μmol/g/h, OLZ),[15] we assumed that an experimental animal (0.25 kg of body weight) had 3 g of cecum contents and a maximum residence time of 10 h (corresponding to 24.9 μmol of azo bond reduction capability); thus, an oral dosage of 0.1 mmol equiv of OLZ/kg was selected for the *in vivo* investigation of rats.

Plasma concentration profiles after the oral administration of OLZ or PEG–OLZ copolymers

After the reduction of azo bonds and hydrolysis splitting of PEG–OLZ copolymers in the intestinal lumen, 5-ASA and its main metabolite ace-5-ASA may be absorbed in the intestine of rats and should be detectable in plasma concentrations. However, in our study, no 5-ASA or metabolites above the limit of quantification could be measured at any sampling point by HPLC.

Knoll et al.[7] reported a comparable horse plasma maximum concentration of ace-5-ASA of about 2 μg/mL after the oral administration of 30 mg of OLZ/kg of body weight to horses and an elimination half-life of about 5.1 h. In our study, no 5-ASA or ace-5-ASA above the limit of quantification could be measured at any sampling point by HPLC in the *in vivo* study. It is believed that the observable differences in the animals and metabolism methods employed by the two studies caused the different results. Rats and horses as test animals will produce differences in the gastrointestinal transit time and in the intestinal microflora activity. By the reduction and hydrolysis of PEG–OLZ copolymers in the lower intestinal lumen, the released 5-ASA and its main metabolite ace-5-ASA might be poorly absorbed in the intestine of rats.

Recovery of 5-ASA and ace-5-ASA in urine and feces after the oral administration of OLZ or PEG–OLZ copolymers

5-ASA and its metabolites in the urine and feces were determined after 96 h of oral administration of OLZ or PEG–OLZ copolymers, respectively. Mean values of the drug concentrations were calculated by the averaging of the corresponding urine and feces concentrations during sampling periods of 4 h each.

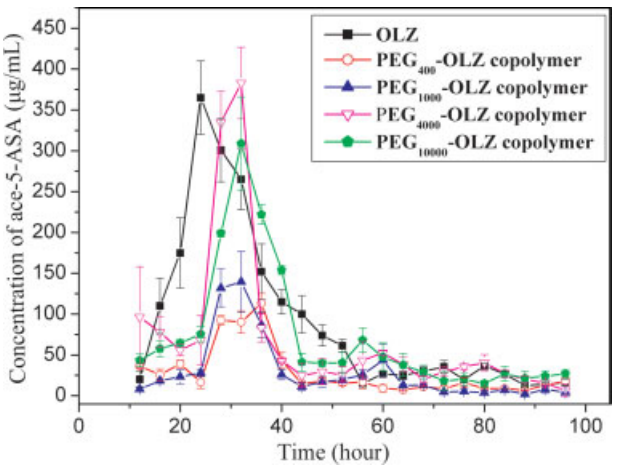

Figure 5 Concentrations of 5-ASA and metabolites in the urine of male S–D rats after the oral administration of OLZ and PEG–OLZ copolymers at 0.1 mmol equiv of OLZ/kg of body weight, respectively. 5-ASA and metabolites were converted to ace-5-ASA. Data are presented at the mean ± standard error (n = 5). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Figure 5 shows the concentrations of 5-ASA and ace-5-ASA in the urine of rats as a function of time after oral administration of OLZ or PEG–OLZ copolymers. It is demonstrated that no ace-5-ASA could be detected within the initial 8 h. Generally, free 5-ASA is rapidly and completely absorbed in the upper intestine when it is administered orally, but it is poorly absorbed in the colon,[23] and then it would be detected in the urine of rats after the oral administration of 5-ASA within 8 h. However, in this study, after the oral administration of OLZ or PEG–OLZ copolymers, the time for 5-ASA and its metabolite in the urine of rats reaching the detectable level was more than 8 h. The data showed that little 5-ASA was released and absorbed in the upper parts of the gastrointestinal tract (stomach and small intestine) of rats. The *in vivo* results were in agreement with the results of the *in vitro* release experiment, showing that PEG–OLZ copolymers were transported mainly intact to the large intestine where azoreductase was present in a high concentration. At the colon site, PEG–OLZ copolymers would be reduced in company with the hydrolysis cleavage of ester bonds in the copolymers, and 5-ASA should be released.

In the time after oral administration, the concentrations of 5-ASA and ace-5-ASA in the urine of rats increased quickly and arrived at the maximum concentrations of 365.0 μg/mL at about 24 h for OLZ, 113.4 μg/mL at 36 h for PEG_{400}–OLZ, and 139.5 μg/mL for PEG_{1000}–OLZ, 383.0 μg/mL for PEG_{4000}–

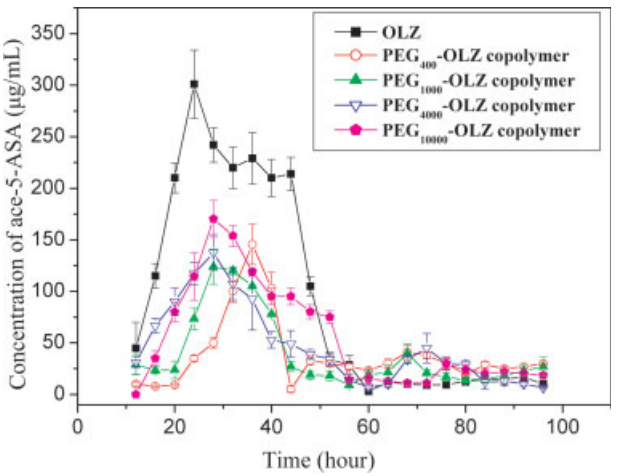

Figure 6 Concentrations of 5-ASA and metabolites in feces water of male S–D rats after the oral administration of OLZ and PEG–OLZ copolymers at 0.1 mmol equiv of OLZ/kg of body weight, respectively. 5-ASA and metabolites were converted to ace-5-ASA. Data are presented as the mean ± standard error (n = 5). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

OLZ, and 309.0 μg/mL for PEG_{10000}–OLZ at 32 h after administration. The concentration of 5-ASA and ace-5-ASA declined gradually thereafter from 56 to 96 h after administration and was no more than 30 μg/mL. The different maximum concentrations and the time points of the maximum concentrations are probably due to the different degradation rates of OLZ and PEG–OLZ copolymers in the colon. OLZ is the low-molecular-weight prodrug, and 5-ASA can be absorbed after the reduction of azo bonds in the lower gastrointestinal tract. Compared with OLZ, PEG–OLZ copolymers must undergo azo bond splitting by bacterial azoreductase and ester bond splitting by hydrolysis in the lower gastrointestinal tract, and then they release 5-ASA. The results also indicated that the PEG–OLZ copolymers had a different degradation activity that was mainly attributed to the difference in their hydrophilicity. With the molecular weight of the PEG segment increasing, the hydrophilicity of the copolymer increased. For instance, PEG_{400}–OLZ and PEG_{1000}–OLZ copolymers dissolved poorly in water, whereas the azo copolymers of PEG_{4000}–OLZ and PEG_{10000}–OLZ were soluble in water. Therefore, the state of the oral materials (particulates and liquids) apparently affected the maximum concentrations of 5-ASA and its metabolites as well as the time of the maximum concentration. Moreover, the materials in the liquid state transiting the gastrointestinal lumen was faster than that in the solid state.[24] Therefore, the degradation rates of the copolymers in the solid state were significantly depressed. In other words, the degradability of the PEG–OLZ copolymers in the gastrointestinal tract of S–D rats can be adjusted by the modulation of the molecular weight of the PEG segments.

The changes in the concentration of 5-ASA and ace-5-ASA in feces samples were similar to the changes in the urine samples after the oral administration of OLZ or PEG–OLZ copolymers. Similarly, it was difficult to detect 5-ASA and ace-5-ASA in the fecal water samples within 8 h. The values reached a maximum concentration of 301.6 μg/mL at 24 h for OLZ (Fig. 6). The mean maximum concentration of ace-5-ASA was 145.5 μg/mL at 36 h for the PEG_{400}–OLZ copolymer, 124.0 μg/mL at 32 h for the PEG_{1000}–OLZ copolymer, 137.5 μg/mL at 28 h for the PEG_{4000}–OLZ copolymer, and 170.30 μg/mL at 28 h for the PEG_{10000}–OLZ copolymer (Fig. 6). However, because of the faster degradation rate of OLZ in the colon, the detected concentration of 5-ASA and ace-5-ASA was lower for OLZ than for PEG–OLZ copolymers between 60 and 96 h. This may be due to the fact that 5-ASA was released more slowly in the lower gastrointestinal tract from the copolymers than from OLZ. That is, the residual time of the copolymers in the colon was relatively long, and the copolymers might have degraded more slowly with the PEG molecular weight decreasing. This demonstrates further the dependence of the molecular structure on the degradability of the copolymers.

Figure 7 present rats' urinary and fecal recovery of 5-ASA and its metabolites after oral administration with OLZ and PEG_{400}–OLZ, PEG_{1000}–OLZ, PEG_{4000}–OLZ, and PEG_{10000}–OLZ copolymers. The accounts calculated by cumulative summation of the excreted amounts of 5-ASA and metabolites in fecal water were 20.9, 10.2, 7.5, 16.2, and 15.0% of the dose, respectively. In urine, recoveries of 5-ASA were 6.9, 3.5, 5.8, 11.7 and 14.5% of the dose, respectively. The total recoveries of urine and feces of OLZ

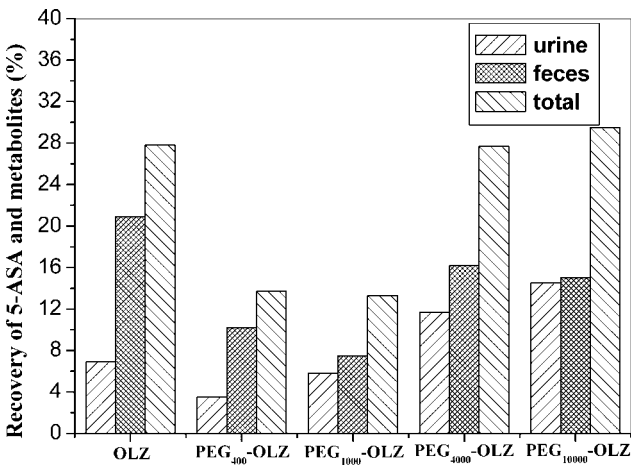

Figure 7 Recovery of 5-ASA and metabolites after the oral administration of OLZ or PEG–OLZ copolymers (0.1 mmol equiv of OLZ/kg of body weight) to male S–D rats. 5-ASA and metabolites were converted to ace-5-ASA.

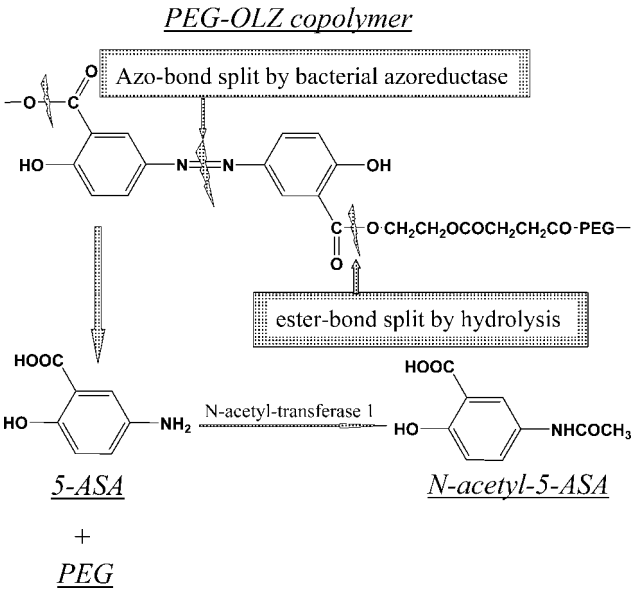

Figure 8 Biological degradation of the PEG–OLZ copolymer.

and PEG–OLZ copolymers were relatively low. This could be the result of the formation of additional metabolic products in the rats by, for example, colonic bacteria, which could not be quantified by our analytical methods, leading to the underestimation of the total recovery. Although there were variations among individual animals, similar trends were shown, and a large percentage of total urinary and feces excretion of 5-ASA and its metabolites (ca. 70%) took place between 16 and 56 h after the oral administration of PEG–OLZ copolymers.

On the basis of an *in vivo* test, the modulation of the PEG weight in PEG–OLZ copolymers can probably improve or alter the rate and location of drug release. 5-ASA could be released in a sustained fashion in the lower gastrointestinal tract through azo bonds cleaved by azoreductase and ester bonds cleaved by hydrolysis (Fig. 8). The PEG segment, as the main degradation product from the hydrolysis of ester bonds, is biocompatible and has low toxicity.[25] In summary, colon-specific drug delivery systems can be developed on the basis of biocompatible PEG and the unique azoreductase activity of the colonic microflora. The resultant PEO–OLZ copolymers appear to be very good candidates as polymeric drugs for colon-specific drug delivery.

CONCLUSIONS

A novel type of linear copolymer composed of PEG with OLZ was developed for colon-specific drug delivery of 5-ASA. 5-ASA was released in a sustained fashion by the reduction of azo bonds and in company with the hydrolysis of ester bonds of the azo copolymers in a medium of cecum contents *in vitro*. In an *In vivo* test, there was 8 h of lag time before 5-ASA could be detected in urine samples, and this indicated that the conjugate could remain intact in the upper part of the gastrointestinal tract. Therefore, PEG–OLZ copolymers could mainly be transported intact to the large intestine, where they were transformed effectively to 5-ASA under the conjunct process of enzyme reduction and hydrolyzation cleavage. Besides, slow elimination of the metabolite in the urine and feces of rats provided a sign of the sustained release in the colon after oral administration of PEG–OLZ copolymers. We could adjust the degradability of PEG–OLZ copolymers in the gastrointestinal tract of S–D rats by modulating the molecular weight of the PEG segments. Because of the advantages of these PEG–OLZ copolymers, it could be concluded that PEG–OLZ copolymers could be promising candidates for colon-specific polymeric prodrugs of 5-ASA.

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