Synthesis and Antitumor Activity of Poly(L-cysteine) Bonded Covalently 5-Fluorouracil

Fushun YANG* and Renxi ZHUO

Department of Chemistry, Wuhan University,
Wuhan 430072, People's Republic of China

(Received January 5, 1990)

ABSTRACT: Poly(L-cysteine) linked covalently 5-fluorouracil was prepared. Hydrolytic release of 5-fluorouracil in alkali and neutral media, and antitumor activity of these new polymeric drugs against Ehrlich ascites carcinoma *in vitro* and *in vivo* were studied.

KEY WORDS Polymeric Carrier Drug / Antitumor Activity / 5-Fluorouracil /

The medicinal chemists often use the term "prodrug" in their literatures. It normally means a biologically active unit that is covalently combined with a physiologically inactive segment. The prodrug, after administration into the system, undergo enzymatic degradation or hydrolysis leading to the generation of the original pharmacon which can then react with the receptor. The latent potentiality of this type of drug has not been fully exploited and perhaps polymer carrier drugs with many facets similar to prodrugs may be developed. In polymeric carrier drugs, the physiological response that is normally observed is primarily due to the reaction of the active pharmacon, released *via* enzymatic hydrolysis or degradation, at the appropriate receptor site.

Very recently, a great deal of interest has centered on the idea of utilizing biocompatible polymers to regulate the delivery of pharmaceuticals and veterinary drugs. So far, a number of polymer-controlled drug delivery systems have been successfully developed to control the rate of drug administration and prolong the duration of therapeutic action as well as to

target the delivery of drugs. Poly(amino acid)s have been used as polymeric carriers to prepare controlled delivery systems, due to their biocompatibility and biodegradable feature.

5-Fluorouracil (5-FU) possess good antitumor activity, but has significantly high toxic side effects. It was reported that 5-FU was covalently linked with poly(L-lysine)¹ and poly(L-tyrosine)² as a means of achieving increased drug uptake by tumor cells and reducing its side effects. In this paper, a series of poly(L-cysteine) (polyCysH) attached 5-FU were prepared in order to restrict the concentration of the drug agent to a narrow range to elicit the desired medical activity while reducing potentially dangerous side effects. The hydrolysis studies in various media, as expected, showed that the polyCysH linked 5-FU *via* an amide link could release the corresponding drug agent, but those *via* a C–N bond could not. Furthermore, the polymeric drugs which can release 5-FU have better antitumor activity against Ehrlich ascites carcinoma.

* To whom all correspondence should be addressed.

EXPERIMENTAL

Reagents and Instruments

L-Cysteine was biochemical reagent. 1-(3-Bromopropionyl)-5-fluorouracil(a),³ 1-chlorocarbonyl-5-fluorouracil(b),⁴ 1-(4-bromobutyl)-5-fluorouracil(c)⁵ and 1-(6-bromohexyl)-5-fluorouracil(d)⁵ were prepared according to reported methods. All other reagents were of AR grade and away off oxygen with ultrasonic waves.

Elemental analysis were determined with Carlo Erba Elemental Analyzer modle 1106. IR spectra were recorded on a Nicolet 170sx Spectrometer. UV spectra were run with a Shimadzu UV-240 Spectrometer. ¹H NMR spectra were recorded on a Varian EM-360L instrument. The number-average molecular weight of polyCysH was determined with the method of VPO on Knauer Osmometer.

Synthesis

Poly(L-cysteine). To a solution of S-carbobenzoxy-N-carboxy-L-cysteine anhydride⁶ (8.0 g) in dry benzene (150 ml) was added dieth-

ylamine (1.0 ml, of a 1% w/v solution in benzene) and the mixture kept at 50°C for 48h. Moisture was excluded by means of a calcium chloride tube, and then petroleum ether (700 ml) was added and the polymer was collected, washed with petroleum ether and dried *in vacuo*, yield 6.2 g (94%).

Anal. Calcd. for (C₁₁H₁₁NO₃S)_n: C, 55.70%; H, 4.64%; N, 5.91%, Found: C, 55.67%; H, 4.70%; N, 6.05%.

Poly(S-carbobenzoxy-L-cysteine) (5.6 g) prepared as above in liquid ammonia (500 ml), finely cut sodium (3.5 g) was added in small portions with magnetic stirring. After three hours, the ammonia was allowed to evaporate and the residue was dissolved in air-free water. The aqueous solution was washed with ether and acidified with a few drops of concentrated hydrochloric acid. The poly(L-cysteine) which separated was collected by filtration under nitrogen pressure, washed with air-free water, and dried in a stream of nitrogen over phosphorus pentoxide, yield 1.4 g (58%). The number-average molecular weight was 2×10^3 (PC₁).

Anal. Calcd. for (C₃H₅NOS)_n: C, 34.05%;

H, 4.85%; N, 13.59%, Found: C, 35.02%; H, 4.97%; N, 13.61%.

Poly(L-cysteine) with molecular weight 3.1×10^4 (PC₂) and 5.6×10^4 (PC₃) were prepared as above-mentioned method by adding 0.5 ml and 0.2 ml diethylamine (1% w/v solution in benzene) into the polymerization solution of *S*-carbobenzoxy-*N*-carboxy-L-cysteine anhydride, respectively.

Poly(L-cysteine) containing 5-FU (PC_{1a} as an example) PolyCysH (0.3 g) and 1-(3-bromopropionyl)-5-fluorouracil (1.5 g) were added to oxygen-free pyridine (20 ml). The mixture was kept at 50°C for 8 h with magnetic stirring, then concentrated to about 10 ml *in vacuo* on a rotatory evaporator. To the residue was added 50 ml of petroleum ether. A precipitate was collected by filtration, washed with water, acetone and petroleum ether, dried under reduced pressure, yield 0.64 g (63.0%, calculated in conversion of S-H group).

Hydrolysis Studies

Hydrolytic release of 5-FU from its matrix was determined by the measurement of UV absorbance of the polymeric drug solution in 267 nm at 37°C at regular interval, compared

with the standard line of 5-FU in the same solvent. The results are shown in Figures 4–6.

RESULTS AND DISCUSSION

Table I shows that the conversion of S-H group, calculated from the nitrogen content analysis, is identical with corresponding reaction yield, which presents a substitution between S-H group in polyCysH and 5-FU derivatives. From Figure 1, it is clear that UV

Figure 1. UV absorption of polyCys-5-FU conjugates (solvent, DMSO; conc, 0.0010 g/ml; temp, 25°C).

Table I. Relation of S-H conversion to reaction conditions and molecular weight of polyCysH

No.	MW of polyCysH ($\times 10^3$)	Deriv. of 5-FU	Reaction conditions			Yield %	S-H conversion ^b %
			R ^a	T/°C	t/h		
PC _{1a}	2.0	a	2	50	8	63.7	64.2
PC _{2a}	31	a	2	50	8	60.6	60.8
PC _{3a}	56	a	2	50	8	54.3	55.0
PC _{1b}	2.0	b	1	40	6	89.5	89.7
PC _{2b}	31	b	1	40	6	85.7	86.1
PC _{3b}	56	b	1	40	6	79.9	80.4
PC _{1c}	2.0	c	2	50	8	53.6	54.3
PC _{2c}	31	c	2	50	8	49.1	50.2
PC _{3c}	56	c	2	50	8	46.8	47.0
PC _{1d}	2.0	d	2	50	8	47.6	48.2
PC _{2d}	31	d	2	50	8	45.3	45.6
PC _{3d}	56	d	2	50	8	40.7	41.0

^a Ratio of moles of 5-FU derivatives to moles of L-cysteine unit in polyCysH.

^b Calculated from the data of nitrogen content analysis.

Figure 2. ^1H NMR of polyCysH and polyCys-5-FU conjugates ($\text{F}_3\text{CCO}_2\text{H}$).

Figure 3. IR spectra of polyCysH and polyCys-5-FU conjugates (KBr).

absorption peaks of aromatic rings appear at about 267 nm, and from Figure 2, the chemical shift (8.25 ppm) of hydrogen on C_6 in 5-FU. In addition, the absorption peaks at 1680 cm^{-1} become significantly strengthened and those at 2550 cm^{-1} (S-H stretched resonance) weakened in IR of polyCysH-5-FU conjugates compared with that of polyCysH.

Figures 4–6 show the delivery of 5-FU from its matrix in alkali and neutral media. As expected, hydrolytic rate of 5-FU from polyCysH-5-FU conjugates *via* an amide link

Figure 4. Hydrolytic delivery of 5-FU from polymeric drugs at 37°C and pH 9 (●, PC_{1c} ; ◐, PC_{1a} ; ○, PC_{1b}).

is faster than that of those *via* a C–N link. From these figures, it is also evident that hydrolysis of polyCysH attached 5-FU *via* the amide link is considerably faster in alkali medium than that in neutral medium.

In general, polymer-controlled drug delivery systems exist medical action only when they release drug units which are fixed to unactive polymeric carrier. In our prepared polymeric drugs, 5-FU is a well-known antitumor agent polyCysH has no antitumor activity. The antitumor activity against Ehrlich ascites

Table II. Antitumor activity of polyCysH fixed 5-FU against Ehrlich ascites acricronoma *in vitro*^a

No.	PC _{1a}	PC _{2a}	PC _{3a}	PC _{1b}	PC _{2b}	PC _{3b}	PC _{1c}	PC _{2c}	PC _{3c}	PC _{1d}	PC _{2d}	PC _{3d}	5-FU	
Concentration mg ml ⁻¹	25	25	25	20	20	20	30	30	30	35	35	35	15	
Inhibition %	4 h	65	60	60	85	76	80	10	8	8	5	5	5	100
	24 h	100	100	100	100	100	100	40	37	35	37	35	35	

^a Tumor cell concentration, 2×10^6 cells/ml; blank test, 1% NaCl solution, 4 h, 4%; 24 h, 15%.

Figure 5. Hydrolytic delivery of 5-FU from polymeric drugs at 37°C and pH 9 (●, PC_{3c}; ◐, PC_{3a}; ○, PC_{3b}). **Figure 6.** Hydrolytic delivery of 5-FU from polymeric drugs at 37°C and pH 7 (●, PC_{1c}; ◐, PC_{1a}; ○, PC_{1b}).

carcinoma *in vitro* (Table II) and *in vivo* (Table III) show that the polymeric drugs attached 5-FU *via* the amide link provide better antitumor activity than those *via* the C–N link, due to hydrolysis and/or enzymatic release of 5-FU from its polymeric carrier. From Tables

Table III. Antitumor activity of polyCysH fixed 5-FU against Ehrlich ascites carcinoma *in vivo*

No.	Dose/ mg kg ⁻¹	Feeding times ^a	T/C ^b /%	Inhibition ratio/% ^c
PC _{1a}	0.600	ip × 3	142	54
PC _{2a}	0.650	ip × 3	142	54
PC _{3a}	0.700	ip × 3	150	57
PC _{1b}	0.500	ip × 3	125	64
PC _{2b}	0.530	ip × 3	130	61
PC _{3b}	0.558	ip × 3	134	59
PC _{1c}	0.700	ip × 3	167	43
PC _{2c}	0.750	ip × 3	167	42
PC _{3c}	0.810	ip × 3	167	42
PC _{1d}	0.800	ip × 3	167	45
PC _{2d}	0.870	ip × 3	167	42
PC _{3d}	0.940	ip × 3	167	43
5-FU	0.260	ip × 3	117	84

^a The mice were injected on 0, 4, and 8 days, respectively.

^b Ratio of median survival of treated mice (T) to that of controlled mice (C).

^c Calculated from formula $(W_1 - W_2)/W_1$, where the W_1 expresses the median increased weight of controlled and the W_2 that of treated mice.

II and III, it is evident that the inhibition of polymeric drugs on Ehrlich ascites carcinoma shows little influence on the number-average molecular weight of polyCysH. In addition, it is supposed that L-cysteine-S-(CH₂)_x-5-FU ($x=4, 6$) present some antitumor activity for the result of effective inhibition of polyCysH attached 5-FU *via* the C–N link.

Acknowledgment. The authors wish to express their thanks to Mr. Zongjie Shi (Hubei

Cancer Institute, Wuhan 430072, P. R. China)
for the test of antitumor activity.

REFERENCES

1. R. Zhuo, C. Fan, and J. Liao, International Symposium on Polymeric Biomaterials, Preprints, Kunming, PRC, 1988, p 169.
2. F. Yang and R. Zhuo, *Chem. J. of Chinese Univ.*, to be published.
3. C. Zhou and F. Yang, *Chem. J. of Chinese Univ.*, **3**(1), 90 (1987).
4. S. Ozaki, H. Mizuno, and H. Mori, Jpn. Kokai, 77-139080; *Chem. Abstr.*, **88**, 105403d (1978).
5. Nian'en Zhou, Doctoral Paper of Wuhan University, 1988.
6. A. Gerger, J. Noguchi, and E. Katchalski, *J. Am. Chem. Soc.*, **78**, 4483 (1956).