NOTES

Synthesis and Antitumor Activity of Polymeric Drugs Containing 5-Fluorouracil in the Main Chain

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5-Fluorouracil (5-FU) has been used for a long time as an antitumor agent. But it has strong toxic side effects. It is expected that the introduction of 5-FU into a macromolecule can reduce the side effects and has a prolonged duration of activity. Many works were reported on the polymeric drug containing 5-FU as the pendant group.¹⁻⁵ As an alternative way, we have reported a series of new polymeric drugs in which 5-FU was linked *via* C-N bond.⁶ These systems are capable of releasing 5-FU derivatives under mild condition. This paper deals with the synthesis, hydrolysis and anti-tumor activity of the polymeric drugs containing 5-FU linked *via* carbonyl groups in the main chain. The synthetic scheme is as follows:

EXPERIMENTAL

Synthesis

1,3-Bis(bromoacetyl)-5-fluorouracil (II) Bromoacetyl bromide (29.6 g, 0.146 mol) in 20 ml of acetonitrile was added dropwise into 2,4-bis(trimethylsilyl)-5-fluorouracil (I) (20.0 g, 0.073 mol)⁷ in 100 ml acetonitrile. Stirred at room temperature for 3.5 hour, acetonitrile was distilled off under reduced pressure. The oily residue was washed with *n*-hexane for 2 times (50 ml $\times$ 2) and recrystallized from benzene. Colorless needle crystals were obtained. Yield 18.0 g (98.4%), mp 88—89°C. $\text{C}_8\text{H}_5\text{O}_4\text{N}_2\text{Br}_2\text{F}$, Found: C 26.05, H 1.51, N 7.43. Calcd for: C 25.81, H 1.34, N 7.53 IR (KBr): 1723, 1703, 1671 cm^{-1} ($\text{C}=\text{O}$), 1247 cm^{-1} (C-F). ^1H NMR (60 MHz, DMSO-

d_6): δ 4.52 ppm (s, 2H, $\text{>N}^1\text{-C(=O)-CH}_2\text{-}$), 4.82 ppm (s, 2H, $\text{>N}^3\text{-C(=O)-CH}_2\text{-}$), 8.32—8.42 (d, 1H, 6-H of pyrimidine ring). UV (Ethanol): λ_{max} 220.6 nm ($\epsilon=4100$), 263.5 nm ($\epsilon=6800$). MS: m/e 372 (M^+).

Poly[ethylene diamino 1,3-bis(methyl carbonyl)-5-fluorouracil] (IIIa) To a mixture of II (37.2 g, 0.01 mol) and NaHCO_3 (1.68 g, 0.02 mol) in 30 ml DMF, ethylene diamine (0.60 g,

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Table I. Experimental data the polymers (**IIIa–e**)

Polymers	Yield %	$\bar{M}_n^a$	Elemental Anal./%					
			Found			Calcd ^b		
			C	N	H	C	N	H
IIIa	74.1	2270	38.87	18.64	5.20	39.21	18.30	4.90
IIIb	88.0	2230	41.49	17.82	5.74	41.25	17.50	5.31
IIIc	83.8	2150	43.33	17.05	5.91	43.11	16.77	5.69
IIId	80.1	1850	45.13	16.38	6.32	44.83	16.09	6.03
IIIe	61.3	1860	46.62	15.78	6.67	46.41	15.47	6.35

^a Determined by vapour phase osmometry (MeOH, 45°C).^b Calculated from $-\text{NH}-(\text{CH}_2)_n-\text{NH}-\text{CH}_2-\text{C}(=\text{O})-\text{N}(\text{F})-\text{C}(=\text{O})-\text{N}(\text{CH}_2)_2-\text{C}(=\text{O})-$ $\cdot 2\text{H}_2\text{O}$.**Table II.** Antitumor activity of polymeric drugs (**IIIa–e**) tested against EAC *in vitro*^a

Compounds and polymers	Inhibition ratio/% Dose/ $\mu\text{g ml}^{-1}$		
	1000	100	10
IIIa	47.1	41.7	35.2
IIIb	55.7	40.5	26.5
IIIc	23.4	44.5	26.7
IIId	44.4	39.0	32.9
IIIe	31.7	35.8	40.4
5-FU	62.4	26.8	7.4

^a Tumor cell concentration: $1-4 \times 10^4$ cells ml^{-1} ; Blank test: 1% NaCl aqueous solution; 4h.

Inhibition ratio (%) =

$$\left(1 - \frac{\text{Survival cell number treated}}{\text{Survival cell number of control}}\right) \times 100$$

0.01 mol) was added. It was allowed to stir at 70–80°C for 24 h and then filtered. The filtrate was poured with vigorously stirring into 500 ml of acetone to precipitate the polymer. The precipitate was then dissolved in 10 ml of *N,N'*-dimethylformamide (DMF). Reprecipitation from 200 ml of acetone to give the product (**IIIa**), 2.0 g, yield 74.1%.

Polymers (**IIIb–e**) were synthesized with the same method as for **IIIa** and the results were shown in Table I.

Hydrolysis of the Polymers

The hydrolysis of polymers were investigated *in vitro* at 37°C under shaking in various kinds of aq solutions. At a given time, the polymers and their hydrolyzates in 0.3 ml of the mixture were separated with TLC method (kiesel gel GF254 (Merck), eluent; acetone: ethyl ether = 8:2). The hydrolyzate (5FU) was dissolved in 10 ml of 0.1 mol l^{-1} NaOH aq solution, and the amount of 5FU released was determined by using UV spectrum method (5100 of ϵ_{283} for 5FU anion in 0.1 mol l^{-1} NaOH aq soln.).

Measurement of Antitumor Activity

The antitumor activity of the polymers (**IIIa–e**) were tested against Ehrlich ascites carcinoma (EAC) both *in vitro* and *in vivo* according to the typical NCI protocol. The results were shown in Tables II and III.

RESULTS AND DISCUSSION

Synthesis and Characterization of the Compound (**II**) and Polymers (**IIIa–e**)

In our previous paper,⁶ 5-FU was ever introduced *via* C–N bond into the main chain of polymers. These polymeric drugs could be hydrolyzed under mild conditions in aqueous

Table III. Antitumor activity of the polymeric drugs (**IIIa—e**) tested against EAC *in vivo*^a

Polymeric drugs No.	Dose mg kg ⁻¹	Route of Admin.	No. of mice in treatment		Average weight of tumor/g		Inhibition ratio %
			Start	End	Control	Treated	
IIIa	65	s.c 10	10	10	1.214	1.010	16.8
	130	s.c 9	10	10	1.214	0.880	27.5
	390	s.c 9	10	8	1.214	0.765	37.0
IIIb	90	s.c 9	10	9	1.214	1.226	
	180	s.c 9	10	9	1.214	0.920	24.2
	540	s.c 9	10	8	1.214	0.611	49.7
IIIc	60	s.c 10	10	9	1.214	0.703	42.1
	120	s.c 10	10	9	1.214	0.617	49.2
	360	s.c 10	10	4	1.214	0.918	24.4
IIId	75	s.c 10	10	10	1.214	0.623	48.7
	150	s.c 10	10	10	1.214	0.615	50.7
	200	s.c 10	10	10	1.214	0.887	26.9
IIIe	100	s.c 10	10	10	1.214	0.863	28.9
	200	s.c 10	10	9	1.214	0.793	34.6
	400	s.c 10	10	8	1.214	0.941	26.2
5-FU	20	s.c 10	10	8	1.214	0.512	58.0

^a *P* value < 0.05.

$$\text{Inhibition ratio (\%)} = \left(1 - \frac{\text{Average tumor wt in treated mice}}{\text{Average tumor wt in control mice}} \right) \times 100$$

solution. However, the hydrolyzate was 5-FU derivatives, but was not 5-FU itself. When 5-FU was linked directly *via* carbonyl group in the main chain, this kind of polymeric drugs can release 5-FU in the hydrolysis. To obtain the polymeric drugs, compound (**II**) was synthesized with the reaction of 2,4-bis(trimethylsilyl)-5-fluorouracil and two equivalents of bromoacetyl bromide. When they reacted in equal mole ratio, 1-bromoacetyl-5-fluorouracil (**IV**) was the main product. (mp 141—142°C). By the condensation of compound (**II**) with a series of α,ω -diamines in the presence of sodium bicarbonate, polymeric drugs in which 5-FU was linked *via* carbonyl group were prepared. All the compound and polymers were structur-

Figure 1. Release rate of 5-FU from polymer **IIIb** in 0.1 mol l⁻¹ NaOH (□), 0.1 M phosphate buffer (▲), 0.1 mol l⁻¹ HCl (×) and polymer **IIIe** in 0.1 M phosphate buffer (△).

ally confirmed by ¹H NMR, IR and UV spectra as well as elemental analysis. These newly prepared polymeric drugs are water-soluble.

Hydrolysis of the Polymers

In order to evaluate the release behavior of 5FU *in vivo*, the hydrolysis of the polymers was carried out using the method described above. The results were shown in Figure 1. The order of hydrolysis of polymer (IIIb) was as follows: 0.1 mol l^{-1} NaOH aq soln. $> 0.1 \text{ M}$ phosphate buffer soln. $> 0.1 \text{ mol l}^{-1}$ HCl aq soln. When hydrolyzed in 0.1 M phosphate buffer, the release rate of polymer (IIIe) was slower than that of polymer (IIIb). It can be concluded that hydrophilicity has significant effects on the release rate of the polymeric drugs.

Antitumor Activity of Polymeric Drugs (IIIa—e)

As shown in Tables II (*in vitro*) and III (*in vivo*), all the polymeric drugs exhibited significant antitumor activity, although lower than that for free 5-FU. The doses of administration have a marked influence on the inhibition ratio. For polymers (IIIc—e), when the dose increased over a definite value respectively, the inhibition ratio decreased, on the contrary. This phenomenon was also

observed by Ning and his coworkers.⁸

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REFERENCES

1. C. G. Gebelein, W. Baig, R. M. Morgan, and R. Glovwacky, *Polym. Prepr.*, **20**, 600 (1979).
2. T. Ouchi, H. Yuyama, and O. Vogl, *Makromol. Chem., Rapid Commun.*, **6**, 815 (1985).
3. T. Ouchi, K. Hagita, M. Kawashima, and T. Inoi, *J. Bioact. Compat. Polym.*, **3**, 53 (1988).
4. R. X. Zhuo, J. Liao, and C. L. Fan, *Chem. J. Chin. Univ. (Eng.)*, **6**(3), 244 (1990).
5. F. S. Young and R. X. Zhuo, *Polym. J.*, **22**, 592 (1990).
6. R. X. Zhuo, Preprints, Symposium on Polymers, (Chengdu, China, Nov. 14—18, 1989), Part 1, p 18.
7. R. Duschinsky and T. F. Gabriel, US Patent, 3,354,160 (1967).
8. N. S. Ning, H. Sun, and Y. Wang, Proceedings of the National III Symposium of Experiment and Research of Immunodeficient Animals, Shanghai, China, November 1990, p 108.