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Antitumor Activity of 1-Alkylcarbamoyl Derivatives of 5-Fluorouracil against L1210 Leukemia[1]

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Relationship between chemical structure and antitumor activity of various 1-alkylcarbamoyl derivatives of 5-fluorouracil by parenteral and oral administrations was examined in L1210 system. By intraperitoneal administration, in aliphatic derivatives, when carbon chain extended stepwisely, both antitumor activity and toxicity to host animals were reduced gradually, but ratios of reduction in activity and toxicity were different each other. *tert*-Butyl derivative showed the highest therapeutic ratio among the derivatives. However, it was lower than the therapeutic ratio of 5-fluorouracil.

On the other hand, by oral administration, antitumor activity and toxicity to host animals were decreased along the extention of carbon chain in aliphatic derivatives and therapeutic ratios of the compounds having even carbons in their side chain were greater than those of the adjoining compounds having odd carbons. Among them, 1-hexylcarbamoyl-5-fluorouracil showed the highest therapeutic ratio. This compound was considered to be more suitable for treatment by oral administration than 5-fluorouracil, though the latter compound was superior than the former by parenteral administration.

Keywords——antitumor agents; structure-activity relationship; L1210; alkylcarbamoyl derivatives; antimetabolites; oral administration; 5FU; HCFU; fluorinated pyrimidines

5-Fluorouracil[3] has been used clinically for the treatment of various tumors by parenteral administration.[4] But it is clinically rather toxic in oral administration.[5] Recently, 1-(2-tetrahydrofuryl)-5-fluorouracil become to use for clinical treatment by oral administratoin.[6] Advantage of the latter compound is a low toxicity especially to gastro-intestinal tract and easy administration for the maintenance therapy after surgical treatment.[6,7] Disadvantage of the latter compound is a low blood concentration of 5-fluorouracil which is an active metabolite of the compound.[8] Further, this compound does not affect the ribonucleic acid (RNA) biosynthesis of cultured cells *in vitro* unlike 5-fluorouracil.[9] These finding prompted us to investigate the rational design of a structural modification of 5-fluorouracil which would be more easily activated in tumor cells or would maintain higher blood concentration of 5-fluorouracil than 1-(2-tetrahydrofuryl)-5-fluorouracil with lesser toxicity to the host by oral admini-

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2) Location: *Tsukiji 5-1-1, Chuo-ku, Tokyo, 104, Japan.*

3) C. Heidelberger, N.K. Chaudhuri, P. Danneberg, D. Mooren, L. Griesbach, R. Duschinsky, R.J. Schmitzer, E. Pleven, and J. Schneider, *Nature* (London), **179**, 663 (1957).

4) M.J. Cline, "Cancer Chemotherapy," W.B. Saunders Co., Philadelphia, U.S.A., 1971.

5) R.G. Hahn, C.G. Moertel, A.J. Schutt, and H.W. Bruckner, *Cancer*, **35**, 1031 (1975).

6) C. Konda, H. Niitani, N. Sakauchi, A. Suzuki, Y. Sakai, T. Sakano, M. Shimoyama, T. Kitahara, K. Kimura, and S. Kumaoka, *Gan-no-Rinsho*, **19**, 495 (1973).

7) S. Fujimoto, T. Akao, B. Itoh, I. Koshizuka, K. Koyano, Y. Kitsukawa, M. Takahashi, T. Minami, H. Ishigami, M. Miyazaki, and K. Itoh, *Gan-no-Rinsho*, **22**, 471 (1976).

8) H. Fujita, K. Ogawa, T. Sawabe, and K. Kimura, *Gan-no-Rinsho*, **18**, 911 (1972); *Idem, ibid.*, **18**, 917 (1972).

9) S. Fujimoto, T. Akao, B. Itoh, I. Koshizuka, M. Takahashi, T. Minami, T. Miyoshi, K. Koyano, and K. Itoh, *Gan-no-Rinsho*, **20**, 680 (1974).

stration.

1-Acyl- or 1-alkylsulfonyl-derivatives of 5-fluorouracil were first found to be active against L1210 leukemia,[10,11] but these compounds were chemically too labile to apply through the oral route.[10] Among other derivatives of 5-fluorouracil, 1-alkyl derivatives were totally inactive even by parenteral administration.[1] Recently, 1-alkylcarbamoyl derivatives of 5-fluorouracil were found to be active by oral administration.[11] The present paper concerns the relationship between chemical structure and antitumor activity of various 1-alkylcarbamoyl derivatives of 5-fluorouracil by parenteral and oral administrations.

Materials and Methods

Tumor System and Evaluation of Antitumor Activity——Screening procedure was mainly on the methods of National Cancer Institute in U.S.A.[12] Female BDF_1 mice weighing 20+2 g were used. Six mice for each group, either test or control, were implanted with 1×10^5 cells of L1210 leukemia as reported previously.[13] The compound to be tested was administered intraperitoneally or orally once daily for 5 days, starting 24 hr after transplantation. Antitumor activity of the compounds was evaluated by the increase in life-span over controls (ILS=T/C%−100). ILS_{30} (the dose showing 30% increase in life-span), maximum ILS, ILS_{max} (the dose showing maximum ILS), and therapeutic ratio (ILS_{max}/ILS_{30}) were determined.[11] Antitumor activity was graded as −: 0—9, +: 10—19, ++: 20—29, +++: 30 or more of ILS%.

Chemicals——5-Fluorouracil, 1-(2-tetrahydrofuryl)-5-fluorouracil, and 1-alkylcarbamoyl-5-fluorouracil were supplied from Mitsui Pharmaceuticals, Inc., and chemical structures of 1-alkylcarbamoyl derivatives are shown in Table I.

Results and Discussion

Antitumor Activity of the Derivatives of 5-Fluorouracil against L1210 Leukemia by Intraperitoneal Administration

As shown in Table I, all of the 1-alkylcarbamoyl derivatives of 5-fluorouracil was markedly active against L1210. Methyl- and phenyl-derivatives were markedly active and their ILS_{30}s were about 10 mg/kg/day and *tert*-butyl derivative was the most active and its ILS_{30} was 8.4 mg/kg/day. In aliphatic derivatives, when carbon chain extended stepwisely, both

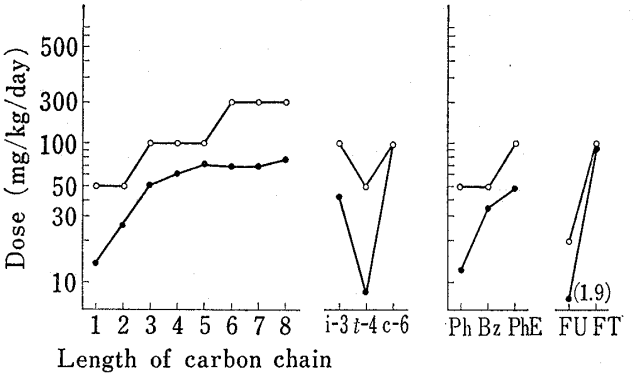


Fig. 1. Relationship between Chemical Structure and Antitumor Activity or Toxicity in 5-Fluorouracil Derivatives by Intraperitoneal Administration

—○—: ILS_{max}, —●—: ILS_{30}, i: iso, *t*: *tert*, c: cyclo, Ph: phenyl, Bz: benzyl, PhE: phenethyl, FU: 5-fluorouracil, FT: 1-(2-tetrahydrofuryl)-5-fluorouracil.

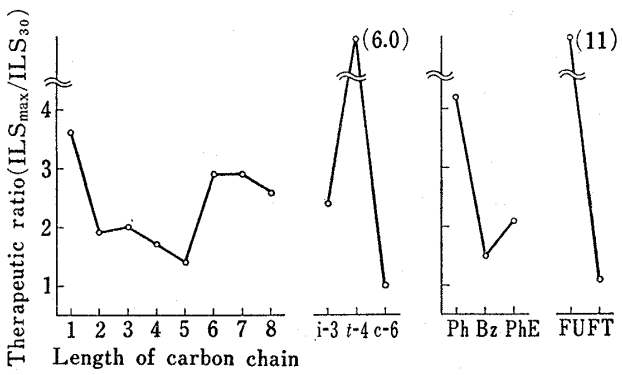
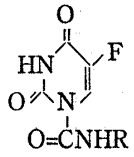


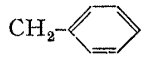
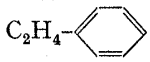
Fig. 2. Relationship between Chemical Structure and Therapeutic Ratio in 5-Fluorouracil Derivatives by Intraperitoneal Administration

i: iso, *t*: *tert*, *c*: cyclo, Ph: phenyl, Bz: benzyl, PhE: phenethyl, FU: 5-fluorouracil, FT: 1-(2-tetrahydrofuryl)-5-fluorouracil.

10) A. Hoshi, M. Iigo, N. Nakamura, and K. Kuretani, *Gann*, **65**, 463 (1974).
11) A. Hoshi, M. Iigo, M. Yoshida, and K. Kuretani, *Gann*, **66**, 673 (1975).
12) Cancer Chemotherapy National Service Center, *Cancer Chemother. Rep.*, **25**, 1 (1962).
13) A. Hoshi and K. Kuretani, *Farumashia*, **9**, 464 (1973).

TABLE I. Antitumor Activity of 5-Fluorouracil Derivatives against L1210 Leukemia by Intraperitoneal Injection

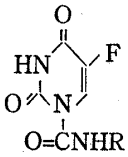


R	Dose (mg/kg/day)	ILS over controls (%)	Antitumor activity
CH_3	100	0	Toxic
	50	65	##
	30	50	##
	10	21	#
C_2H_5	100	−10	Toxic
	50	44	##
	30	33	##
	10	9	−
C_3H_7	200	−3	Toxic
	100	60	##
	50	29	#
	30	14	+
C_4H_9	200	−11	Toxic
	100	56	##
	50	21	#
	30	13	+
C_5H_{11}	300	−6	Toxic
	200	18	+
	100	38	##
	50	21	#
	30	13	+
C_6H_{13}	300	−39	Toxic
	200	54	##
	100	44	##
	30	10	+
C_7H_{15}	300	40	##
	200	48	##
	100	46	##
	30	9	−
C_8H_{17}	300	−12	Toxic
	200	35	##
	100	35	##
	30	13	+
iso-C_3H_7	200	−11	Toxic
	100	78	##
	70	46	##
	30	20	#
tert-C_4H_9	10	16	+
	100	4	##
	50	50	##
	30	40	##
	10	34	##
	3	9	−
cyclo-C_6H_{11}	300	−10	Toxic
	200	0	−
	100	29	#
	50	25	#
	30	16	+
	100	25	#
	50	53	##
	30	48	##
	10	26	#
	100	12	+
	50	44	##
	30	25	#
	300	−19	Toxic
	200	13	+
	100	57	##
	50	31	##
	30	17	+
5-Fluorouracil (FU)	30	60	##
	20	91	##
	10	73	##
	3	38	##
	1	19	+
1-(2-Tetrahydrofuryl)-5-fluorouracil (FT)	200	−2	Toxic
	100	34	##
	70	9	−
	30	0	−

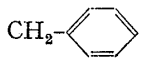
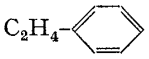
antitumor activity and toxicity to host animals were reduced gradually. However, rates of reduction in activity and toxicity were different each other (Fig. 1). *tert*-Butyl derivatives has the highest therapeutic ratio among the derivatives. Therapeutic ratios of the compounds having 6 or more carbons in aliphatic side chain are greater than those of the compounds having 2 to 5 carbons except *tert*-butyl derivative. In aromatic group, phenyl derivative shows the highest therapeutic ratio (Fig. 2).

Though the therapeutic ratio of *tert*-butyl derivative is the highest among the derivatives, it is still lower than that of 5-fluorouracil by intraperitoneal administration. These results are analyzed with an operational scheme for analog synthesis in drug design proposing by

Table II. Antitumor Activity of 5-Fluorouracil Derivatives against L1210 Leukemia by Oral Administration



R	Dose (mg/kg/day)	ILS over controls (%)	Antitumor activity
CH_3	100	13	+
	50	47	##
	30	33	##
	10	14	+
C_2H_5	100	39	##
	70	62	##
	30	31	##
	10	13	+
C_3H_7	200	10	+
	100	60	##
	70	38	##
	30	16	+
C_4H_9	200	20	#
	100	54	##
	50	38	##
	30	23	#
	10	6	−
C_5H_{11}	300	23	#
	200	41	##
	100	22	#
	30	3	−
C_6H_{13}	300	23	#
	200	53	##
	100	50	##
	50	34	##
	30	21	#
C_7H_{15}	300	21	#
	200	39	##
	100	26	#
	30	23	#
	10	6	−
C_8H_{17}	700	35	##
	500	33	##
	300	31	##
	100	15	+
iso-C_3H_7	200	10	+

R	Dose (mg/kg/day)	ILS over controls (%)	Antitumor activity
	100	44	##
	70	56	##
	30	29	#
	10	13	+
tert-C_4H_9	200	−7	Toxic
	100	54	##
	70	38	##
	30	25	#
	10	10	+
cyclo-C_6H_{11}	300	15	+
	200	59	##
	100	41	##
	30	21	#
	100	14	+
	50	31	##
	30	14	+
	200	23	#
	100	44	##
	70	48	##
	30	29	#
	10	19	+
	500	13	+
	300	33	##
	200	18	+
	100	16	+
5-Fluorouracil (FU)	70	15	+
	50	56	##
	30	33	##
	10	9	−
1-(2-Tetrahydrofuryl)-	200	16	+
5-fluorouracil (FT)	100	31	##
	70	15	+
	30	0	−

Topliss and Martin.[14] However, *tert*-butyl derivative is not the final compound.

Antitumor Activity of the Derivatives of 5-Fluorouracil against L1210 by Oral Administration

In order to evaluate the activity of the compounds in prediction of the effects in clinical use, activity was determined by oral administration, and this route of administration was applied clinically for maintenance therapy after surgical treatment. As shown in Table II, all of the alkylcarbamoyl derivatives of 5-fluorouracil was markedly active against L1210

14) J.G. Topliss and Y.C. Martin, "Drug Design," ed. by E.J. Ariëns, Vol. V, 1, Academic Press, New York, 1975.

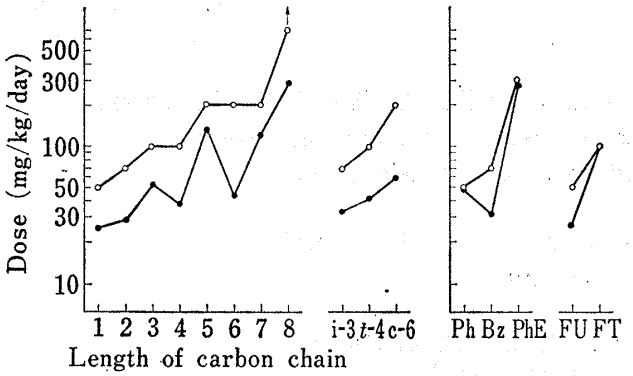


Fig. 3. Relationship between Chemical Structure and Antitumor Activity or Toxicity in 5-Fluorouracil Derivatives by Oral Administration

—○—: ILS_{max}, —●—: ILS_{30}, i: iso, *t*: *tert*, c: cyclo, Ph: phenyl, Bz: benzyl, PhE: phenethyl, FU: 5-fluorouracil, FT: 1-(2-tetrahydrofuryl)-5-fluorouracil.

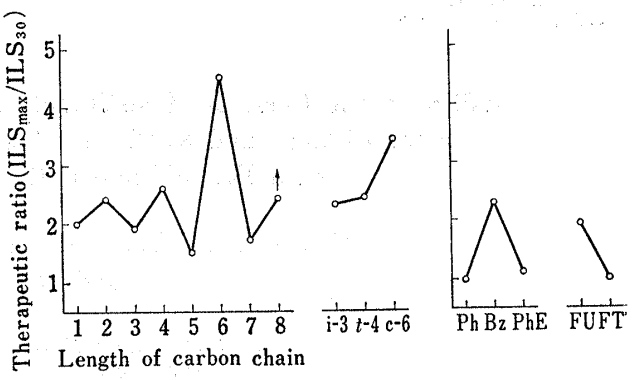


Fig. 4. Relationship between Chemical Structure and Therapeutic Ratio in 5-Fluorouracil Derivatives by Oral Administration

i: iso, *t*: *tert*, c: cyclo, Ph: phenyl, Bz: benzyl, PhE: phenethyl, FU: 5-fluorouracil, FT: 1-(2-tetrahydrofuryl)-5-fluorouracil.

by oral route. In this experiments, antitumor activity and toxicity to host were decreased along the extention of carbon chain (Fig. 3), but therapeutic ratios of the compounds were varied. However, therapeutic ratios of the compounds having even carbons in their side chain are greater than those of the adjoining compounds having odd carbons (Fig. 4). Many compounds in this series show higher therapeutic ratios than both 5-fluorouracil and 1-(2-tetrahydrofuryl)-5-fluorouracil. Among them, 1-hexylcarbamoyl-5-fluorouracil (HCFU) shows the highest therapeutic ratio, that is 4.5. Therapeutic ratio of 5-fluorouracil is decreased by oral administration, while it is markedly active by intraperitoneal one. However, therapeutic ratio of 1-hexylcarbamoyl derivative is higher by oral route than by parenteral one. As a result, this compound was selected as a candidate of antitumor agent. This compound was also markedly active against various tumors such as adenocarcinoma 755, Nakahara-Fukuoka sarcoma, ascites sarcoma 180, Ehrlich ascites carcinoma, C1498 leukemia by oral route as reported previously.[15] These results are also analyzed with an operational scheme for analog synthesis in drug design proposing by Topliss and Martin.[14] This scheme is based on the Hansch method.[16] Therapeutic ratio is used as the index for selection. With this scheme, the most suitable key compound, cyclohexyl derivative can be selected in only three steps: methyl<iso-propyl, cyclohexyl>iso-propyl, and cyclohexyl>benzyl. This compound shows the highest therapeutic ratio among the other key compounds such as ethyl, phenyl, benzyl, *tert*-butyl, and phenethyl derivatives.

The most suitable hexylcarbamoyl derivative has six carbons similar to the selected cyclohexyl derivative with the operational scheme and it can be selected within further few steps of experiments in small groups of compounds. However, if ILS_{30} which is an index for only activity would be used for selection, cyclohexyl derivative could not be selected. As a result, this operational scheme is considered to be valuable for drug design in this case and therapeutic index must be used as a parameter in antitumor agents.

Acknowledgement The authors are indebted to Mitsui Pharmaceuticals, Inc., for kindly supplying the test compounds.

15) A. Hoshi, M. Iigo, A. Nakamura, M. Yoshida, and K. Kuretani, *Gann*, **67**, 725 (1976).
16) C. Hansch, "Drug Design," ed. by E.J. Ariëns, Vol. 1, Academic Press, New York, 1975, p. 271.