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Tumor Inhibitory Effects of a New Fluorouracil Derivative: 5′-Deoxy-5-Fluorouridine

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Abstract—*5′-Deoxy-5-fluorouridine (5-DFUR) has a high cytostatic activity, both intraperitoneally and orally against experimental tumors. It has a particularly strong antitumor effect on the Crocker sarcoma S 180, the Lewis lung carcinoma and a chemically induced squamous cell carcinoma of the skin. Its effect was less marked on the leukemia L 1210 and the B-16 melanoma. The ratio between antitumor activity and toxicity is more favourable than that of 5-fluorouracil, 2′-deoxy-5-fluorouridine and 1-(2-tetrahydrofuryl)-5-fluorouracil.*

INTRODUCTION

5-FLUOROURACIL (5-FU) was the first of a series of pyrimidine antimetabolites possessing a growth inhibitory effect on transplantable tumors [1, 2]. In clinical cancer chemotherapy 5-FU has established itself mainly in the treatment of tumors of the gastrointestinal tract, liver, pancreas, breast and ovary. Considerable efforts have been made towards finding 5-FU derivatives with better antitumor activity and less toxicity. Among these compounds 2′-deoxy-5-fluorouridine (FUDR) [3] and 1-(2-tetrahydrofuryl)-5-fluorouracil (Ftorafur, FT) [4] have been the subject of considerable interest in experimental as well as in clinical cancer chemotherapy. Since the therapeutic results of all these compounds are still unsatisfactory, the search for new 5-FU derivatives is continuing. We are reporting here on the antitumor effects of a new compound, 5′-deoxy-5-fluorouridine (5-DFUR), which, in comparison with 5-FU, FUDR and FT, showed equal or better cytostatic activity at a lower level of toxicity.

MATERIALS AND METHODS

Compounds

5′-Deoxy-5-fluorouridine (5-DFUR) (Fig. 1) was synthesized in the laboratories of Hoffmann–La Roche Inc., Nutley, N.J. by Dr. A. Cook. The compound is a water-soluble crystalline powder. The reference compounds 5-fluorouracil (5-FU), 2′-deoxy-5-fluorouridine (FUDR) and 1-(2-tetrahydrofuryl)-5-fluorouracil (FT) (Fig. 1) are also water-soluble crystalline powders.

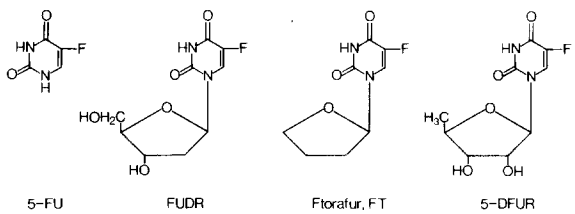

Fig. 1.

Animals and tumors

Experiments were carried out on various experimental tumors in mice: leukemia L 1210 (L 1210), Crocker sarcoma S 180 (S 180), Lewis lung carcinoma (3 LL), B-16 melanoma (B-16) and a dimethylbenz(a)-anthracene (DMBA)-induced skin carcinoma.

Transplantable tumors

In the case of L 1210, 10^5 cells from L 1210 ascites were injected i.p. into BDF_1 mice = F_1-hybrids (DBA × C57BL). S 180 was implanted

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s.c. into Swiss albino mice with 3.5×10^6, 3 LL s.c. into BDF_1 mice with 2.6×10^6 and B-16 i.p. into BDF_1 mice with 2×10^6 viable cells in 0.5 ml tumor homogenate per animal. The viability was determined by the trypan blue exclusion method. Groups of five female mice weighing 22–24 g were used for each dose. Daily administration of the test compounds in aqueous solution was started on the day of implantation and was continued i.p. or p.o. five times a week until death or until one day before tumor excision. S 180 tumors were excised on day 9 and 3 LL tumors on day 23 after implantation. Animals still living on the day of tumor excision were called survivors. A tumor inhibitory effect was evaluated either by comparing the average tumor weight of treated and untreated animals (S 180, 3 LL) and expressing this in per cent of tumor growth inhibition or by comparing the average survival times of treated and control groups (L 1210, B-16). In the latter case the dose eliciting the maximal increase of life span (ILS) was determined.

Toxicity

The LD_{50} was measured using the following method: the drugs were administered i.p. or p.o. to Swiss albino mice (22–25 g) once daily for 5 consecutive days. The LD_{50} values were determined 10 days after the last administration of the drug, to include late toxicity caused mainly by the consequences of bone marrow and gastrointestinal toxicity.

Therapeutic ratio

To determine and to compare the therapeutic ratio i.e., the relationship between antitumor effect and toxicity within this group of pyrimidine antimetabolites, we have chosen as an arbitrary scale the quotient (Q) of the dose necessary to achieve a growth inhibitory effect of 67% ($D\ gi\ 2/3$) in the sarcoma S 180 test and the LD_{50} described above. Thus we calculated the therapeutic ratio as follows:

$$Q = \frac{LD_{50}}{D\ gi\ 2/3}$$

Chemically induced carcinoma

Squamous cell carcinomas of the skin were induced in Swiss albino mice by the method described previously [5]. The shaved skin of the back was painted twice with 7,12-dimethylbenz(a)anthracene in acetone and then repeatedly with croton oil until carcinomas appeared, 5–8 months after commencement of treatment. The experiment was started when the carcinomas had reached a minimal diameter of 12 mm. The mice received 19 i.p. injections of the test compounds within 27 days (5 times a week). The approximate volumes of the carcinomas were determined as the product of crossed diameters × height at the beginning (day 0) and at the end (day 28) of the experiment. The change of volume was expressed as a percentage of the value of day 0. Animals still living at the end of the experiment were called survivors.

RESULTS

Transplantable tumors (Tables 1, 2, 3 and 5)

5'-Deoxy-5-fluorouridine and 2'-deoxy-5-fluorouridine were tested in four transplantable tumors.

Tables 1 and 2 illustrate the strong inhibitory effects of the two compounds on the growth of the solid tumors S 180 and 3LL. The growth inhibition values obtained with 5-DFUR were similar to those achieved with the same doses of FUDR. However, 5-DFUR was much better tolerated than FUDR, as can be seen from the high number of surviving animals at the doses of 100–800 mg/kg of 5-DFUR. The same doses of FUDR were already toxic or lethal.

In the case of the two i.p. transplanted tumors L 1210 and B-16 the effects of the two compounds concerning maximal increase of life span were similar. In L 1210, 5-DFUR in doses of 200 mg/kg i.p. or 400 mg/kg p.o. reached maximal ILS values of 171 and 69% respectively. In the same test FUDR achieved a maximal ILS of 218 and 59% with a dose of 100 mg/kg given i.p. and p.o., respectively. In the B-16 test the maximal ILS was 46% with 400 mg/kg 5-DFUR i.p. and 70% with 50 mg/kg FUDR i.p. The leukemia L 1210 was markedly, the melanoma B-16, however, only moderately influenced by these two compounds.

Table 3 shows a comparison of the effects of 4 pyrimidine antimetabolites on the white blood cell counts and on the growth inhibition of the sarcoma S 180.

Toxicity and therapeutic ratio (Tables 4 and 5)

The LD_{50} values are given in Table 4. These values were further used to calculate the therapeutic ratio (Q).

The therapeutic ratio taking into account antitumor effect and toxicity—Q values (Table 5)—demonstrate a clear superiority of the new compound 5-DFUR, administered

Table 1. *Crocker sarcoma S* 180. *Comparison of the tumor growth inhibition and the number of survivors in mice treated by* 5-*DFUR and FUDR. Test compounds were administered to Swiss albino mice* 6 *times within* 8 days. *Tumor excision and determination of tumor weight on* day 9

		5-DFUR		FUDR	
Daily dose (mg/kg)	Route	Tumor growth inhibition (%)	No. survivors/ No. tested	Tumor growth inhibition (%)	No. survivors/ No. tested
25	i.p.	53.0	5/5	46.8	5/5
	p.o.	41.3	5/5	34.0	5/5
50	i.p.	72.4	5/5	77.0	5/5
	p.o.	76.3	5/5	41.5	5/5
100	i.p.	76.5	5/5	80.0	5/5
	p.o.	91.4	5/5	76.2	3/5
200	i.p.	87.2	5/5	86.3	3/5
	p.o.	94.0	5/5	83.0	3/5
400	i.p.	96.1	5/5		0/5
	p.o.	92.9	5/5		0/5
800	p.o.	98.8	4/5		

Forty controls had a mean tumor weight of 1.125 g ± 0.117 S.E., as calculated from 8 separate experiments.

Table 2. *Lewis Lung carcinoma. Comparison of the tumor growth inhibition and the number of survivors of mice treated by* 5-*DFUR and FUDR. Test compounds were administered* 16 *times within* 22 days *to* BDF_1 *mice. Tumor excision and determination of tumor weight on* day 23

		5-DFUR		FUDR	
Daily dose (mg/kg)	Route	Tumor growth inhibition (%)	No. survivors/ No. tested	Tumor growth inhibition (%)	No. survivors/ No. tested
25	i.p.	32.8	5/5	65.1	5/5
	p.o.	38.0	5/5	55.3	5/5
50	i.p.	58.0	5/5	97.8	5/5
	p.o.	55.6	5/5	84.6	5/5
100	i.p.	93.7	5/5	99.1	5/5
	p.o.	92.9	5/5	96.5	4/5
200	i.p.	100	5/5		0/5
	p.o.	99.5	5/5		0/5
400	p.o.	99.4	5/5		

Twenty controls had a mean tumor weight of 3.44 g ± 0.11 S.E., as calculated from 4 separate experiments.

either i.p. or p.o., over the other pyrimidine derivatives. Its therapeutic ratio is up to 14 times more favourable than that of the other 5-fluorouracil derivatives.

Chemically induced carcinoma (*Table* 6)

5-DFUR, FUDR and 5-FU were also tested in the DMBA-induced skin carcinoma of the mouse (Table 6). Owing to the marked toxicity of FUDR only 5 of 8 animals were alive after 19 i.p. injections of 200 mg/kg. With the well tolerated doses of 100 mg/kg FUDR no tumor regression was observed. 5-FU even at toxic doses showed no tumor regression. With 200 and 400 mg/kg 5-DFUR, however, tumor volume regression of 51 and 86%, respectively, was achieved. The property of 5-DFUR of bringing about a regression

Table 3. Comparison of the effects of 5-DFUR, FUDR, FT and 5-FU on mean white blood cell counts (WBC) after 10 daily administrations and tumor growth inhibition of S 180 after 6 daily administrations of the drugs

Daily dose (mg/kg)	Route	5-DFUR		FUDR		FT		5-FU	
		WBC	Tumor growth inhibition (%)	WBC	Tumor growth inhibition (%)	WBC	Tumor growth inhibition (%)	WBC	Tumor growth inhibition (%)
12.5	i.p.							6000	28.7
25			53.0	4500	46.8			3600	48.4
50			72.4	3400	77.0		12.8	1050	75.0
100			76.5	3100	80.0		37.4		
200		7700	87.2	2950	86.3	3000	61.8		
400		5750	96.1			1850	71.6		
600		3400				900	88.1		
12.5	p.o.								42.0
25			41.3		34.0			5900	60.0
50			76.7	3350	41.5		29.2	1700	68.0
100			91.4	2650	76.2		49.0		
200		7300	94.0	2200	83.0	6500	54.5		
400		3950	92.9			700	75.0		
600		2200							
800			98.8						

Tumor weight: 40 controls had a mean tumor weight of 1.125 g ± 0.117 S.E.
White blood cell count: controls had a mean (± S.E.) white blood cell count of 9370 ± 640/mm³ blood.

Table 4. LD_{50} *values of the 4 compounds tested*

Compound	Route	
	i.p.	p.o.
5-DFUR	550±120*	650±140
FUDR	180±20	180±20
FT	290±30	350±40
5-FU	54±8	88±10

*Values are given in mg/kg and were determined as described in Materials and Methods.

Table 5. Comparison of the therapeutic ratios (Q) *of 5-DFUR, FUDR, FT and 5-FU. The higher the therapeutic quotient, the better is the relationship between antitumor activity and toxicity*

Compound	Route	
	i.p.	p.o.
5-DFUR	$\frac{550}{43}\simeq 13$	$\frac{650}{43}\simeq 15$
FUDR	$\frac{180}{42}\simeq 4.2$	$\frac{180}{85}\simeq 2.1$
FT	$\frac{290}{300}\simeq 1$	$\frac{350}{320}\simeq 1.1$
5-FU	$\frac{54}{43}\simeq 1.3$	$\frac{88}{50}\simeq 1.8$

Therapeutic ratio calculated according to formula:

$$Q=\frac{LD_{50}}{Dgi\,2/3}.$$

of DMBA-induced squamous cell carcinomas of the skin in tolerated doses is in striking contrast to the fact that FUDR, 5-FU and other chemotherapeutic agents such as amethopterin, bleomycin, cyclophosphamide and procarbazine did not lead to regression of this tumor in well tolerated doses [6].

DISCUSSION

The use of chemotherapeutic agents in clinical oncology is limited by their narrow therapeutic margin. Better clinical results can only be expected from compounds possessing a more favourable therapeutic ratio. The fact that the therapeutic quotient of 5-DFUR is—depending on the reference compound and the route of administration—up to 14 times better than that of 5-FU, FUDR and FT (Table 5) gives reason to hope that this compound may also give better therapeutic results in clinical oncology. The ability of 5-DFUR to influence the DMBA-induced squamous cell carcinoma resistant to other cancer chemotherapeutic agents is considered a particularly promising phenomenon. It is very likely that the low bone marrow toxicity contributes markedly to the low general toxicity. As can be seen from Table 3, a strong tumor growth inhibition can be achieved by doses of 5-DFUR causing almost no leukopenia. With the reference compounds, however, the same degree of growth inhibition is accompanied by a marked leukopenia.

The reasons for the high antitumor activity and the low toxicity of 5-DFUR may be

Table 6. DMBA-induced skin carcinoma. Comparison of tumor progression or regression and number of survivors in controls and mice treated with 5-DFUR, FUDR or 5-FU. Mean tumor volume change after 19 *i.p. applications of the test compounds within* 28 days

Compound	Daily dose i.p. (mg/kg)	No. survivors/ No. tested	Mean change of carcinoma volume (%±S.E.)	*P*-values with respect to untreated controls
Controls*		8/8	+287±77.4	—
5-DFUR	200	9/9	−51±14.3	$P<0.005$
5-DFUR	400	8/8	−86± 3.3	$P<0.005$
FUDR	100	10/10	+11±35.8	$P<0.005$
FUDR	200	5/8	−66± 8.0	$P<0.005$
5-FU	20	8/8	+110±34.2	$P<0.025$
5-FU	30	6/8	+23+11.0	$P<0.01$

*Controls (8 animals) had a mean carcinoma volume (±S.E.) of 815±201 mm^3 at the beginning of the experiment.

manifold, and its mechanism of action is not yet fully elucidated. Experiments with *E. coli* and tumor cell cultures [7] have shown that 5-DFUR does not act in the same way as FUDR. Whereas growth inhibition of bacteria or mammalian tumor cells by FUDR can be rescued by thymidine but not by uridine, the contrary is true for 5-DFUR. In investigations of the cellular metabolism, it has been found that 5-DFUR is converted to 5-FU by the enzyme uridine phosphorylase [8], which explains why in cell cultures the effect of 5-DFUR is antagonised by uridine. There is greater activity of uridine phosphorylase in tumor tissues than in normal tissues. In distribution studies [9] a high concentration of 5-FU was found in tumors after the administration of 5-DFUR. Twenty-four hr after the application of 5-DFUR the concentration of 5-FU in the sarcoma S 180 or in the Walker carcinoma was 3–70 times higher than in liver, spleen, small intestine, heart, brain and blood. This phenomenon has not been observed after the administration of 5-FU or ftorafur.

From these investigations the conclusion may be drawn that 5-DFUR probably owes its selective antitumor effect to the high activity of uridine phosphorylase in tumors, where it converts 5-DFUR to 5-FU. The high concentrations of 5-FU in neoplastic tissue compared with the low ones in other tissues may explain the good therapeutic ratio of this compound.

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