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Fluorinated Pyrimidines VI. Effects of 5-Fluorouridine and 5-Fluoro-2'-Deoxyuridine on Transplanted Tumors.*† (23777)

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Descriptions of the tumor-inhibitory properties of 5-fluorouracil and 5-fluoroorotic acid, members of the series of fluorinated pyrimidines developed in a collaborative research between the McArdle Memorial Laboratory of the University of Wisconsin and Hoffmann-La Roche Inc., have previously been reported (1-4). Earlier work at McArdle(5) had demonstrated that uracil-2-C14 was utilized for nucleic acid biosynthesis by Flexner-Jobling carcinoma to a greater extent than by most normal tissues. Accordingly, it appeared likely that an analog closely related to uracil might have a chemotherapeutic potential in the treatment of cancer. Because of the well-known alterations of biological activity produced by the substitution of fluorine for hydrogen in a number of classes of physiologically active compounds and because of the similarity in the Van der Waals radii of the fluorine and hydrogen atoms, a fluorinated uracil was desired. The fluorine atom was placed in the 5-position because the methyl group of thymine is attached to uracil at that locus, and it was anticipated that 5-fluorouracil would block thymine biosynthesis, as indeed is the case(1). As the result of the logical extension of studies of the fluorinated pyrimidine bases, the nucleosides, 5-fluorouridine and 5-fluoro-2'-deoxyuridine, were synthesized by Duschinsky *et al.*(6). This paper reports on the tumor-inhibitory properties of these compounds; some of their biochemical and metabolic effects have been given elsewhere(1,7-9).

Methods. The fluorinated pyrimidines were prepared and supplied by Dr. Robert Duschinsky and his colleagues of Hoffmann-La Roche Inc.(3,6), and were injected intraperitoneally unless otherwise indicated, starting one day after transplantation. The treatment ordinarily involved daily injections for 7 days. Sarcoma 180 and Adenocarcinoma 755 were transplanted by trocar into groups of female Swiss and BDF_1 mice, and the volumes measured at various times after transplantation. The justification for volume measurements and the methods of calculation are presented elsewhere(2). The Ehrlich ascites carcinoma and L-1210 leukemia were carried in ascites form in female Swiss and BDF_1 mice respectively, and the survival times of the mice were recorded. A 5-fluorouracil-resistant line of the Ehrlich ascites carcinoma was also used(2). The Novikoff hepatoma was carried by intraperitoneal transplantation of minced tissue in female Holtzman rats and their survival times were observed.

Results. Toxicity. Some information on the toxicity of 5-fluorouracil has been reported elsewhere(2). At the present time toxicity studies on the nucleosides of 5-fluorouracil have been limited to experiments in albino mice (strain IS 32). Both compounds are characterized by comparatively low toxicities

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† Paper V in the series is reference 9.

when given in a single dose.

With 5-fluorouridine (FUR) the LD_{50} of a single dose was found to be 384 mg/kg by the subcutaneous route, 275 mg/kg by intraperitoneal, and 250 mg/kg by intravenous administration. Toxic doses produced loss of weight (up to 25%) and diarrhea; death occurred not earlier than 6 days and not later than 13 days after a single dose of FUR. Repeated subcutaneous injections required a considerable reduction of the dose. Six injections at 25 mg/kg/day were fatal; the mice succumbed between the 6th and 8th day with a loss of 34% of the starting weight. Experiments in mice implanted with tumors showed that FUR is tolerated only at doses of 4.5 to 5 mg/kg/day for periods of 7-10 days if given subcutaneously or intraperitoneally. 5-Fluoro-2′-deoxyuridine (FUDR) was less toxic. No acute lethal dose was reached by intravenous injection; 1000 mg/kg was tolerated by 3 of 5 mice, and 2 mice died on the 7th day with negligible weight loss. The only sign of toxicity was an extreme loss of hair, which occurred in all mice in the days following the injection and which persisted unchanged during the entire observation period of 14 days. A lethal single dose of FUDR was only attained by intraperitoneal injection; 1000 mg/kg was followed by diarrhea and emaciation and the mice died 6 to 8 days after the injection. A dose of 500 mg/kg was tolerated by 4 out of 5 mice, with one fatality which occurred on the 7th day. Significant weight loss has only been observed in one animal which lost 24% of its initial weight, but was still alive on the 14th day. The LD_{50} by intraperitoneal injection was, therefore, approximately 650 mg/kg. Subcutaneous injection of 1000 mg/kg resulted in a slight loss of body weight (average loss 14%) during the first 6 days. The weights then increased slowly. One out of 5 mice died on the 7th day, another on the 12th day. However, it is uncertain whether the deaths were caused by FUDR. Repeated subcutaneous injections of 100 mg/kg given once daily on 6 successive days were well tolerated; initial weight loss was negligible and was followed by normal gains. Up to 15 intraperitoneal doses of 50 mg/kg were found to be without toxic effects in mice implanted with tumors.

Microscopic examination of sections of the organs of mice having received fatal doses of FUR revealed as the most prominent pathological changes extensive hemorrhages in the lungs and severe hemorrhages in the sternal bone marrow. After administration of single doses (1000 mg/kg) of FUDR similar but less extensive changes were observed infrequently. One mouse which was sacrificed 3 days after the last of 6 subcutaneous doses of 100 mg/kg of FUDR was free of these lesions.

Sarcoma 180. The effects of the fluorinated pyrimidines on the growth of this tumor are shown in Fig. 1, where the survival, tumor volume in mm^3, and weight change are plotted against days after transplantation. The abbreviations used are: 5-fluorouracil (FU), 5-fluorouridine (FUR), and 5-fluoro-2′-deoxyuridine (FUDR). The number of mice in each group are given in the parenthesis, and the dose is expressed as mg/kg/no. of days. It will be noted that in the case of 5-fluorouridine (FUR) a dose of 4.5 mg/kg/7 days caused a considerable weight loss, but no significant effect on the growth rate of the tumor. The deoxyriboside (FUDR) at a dose of 50 mg/kg/7 days when given by stomach tube (S.T.) was also ineffective. The small numbers of mice in the FUDR groups was necessitated by a limitation of supply of the compound. It will be observed that FUDR when given intraperitoneally at a dose of 50 mg/kg/7 days was more effective and produced less weight loss than an equimolar dose of FU (25 mg/kg/7 days). The numbers on the graph at each point represent the fraction of the tumor volume as compared to untreated controls. When FUDR was given by intraperitoneal injection at 50 mg/kg/14 days the tumors did not appear.

In a duplicate experiment, in which FUDR was given to a group of 6 mice by intraperitoneal injection of 50 mg/kg for a period of 15 days, no tumors developed during the treatment period and no growth was observed during 12 days following discontinuance of the drug injections. Three of the animals re-

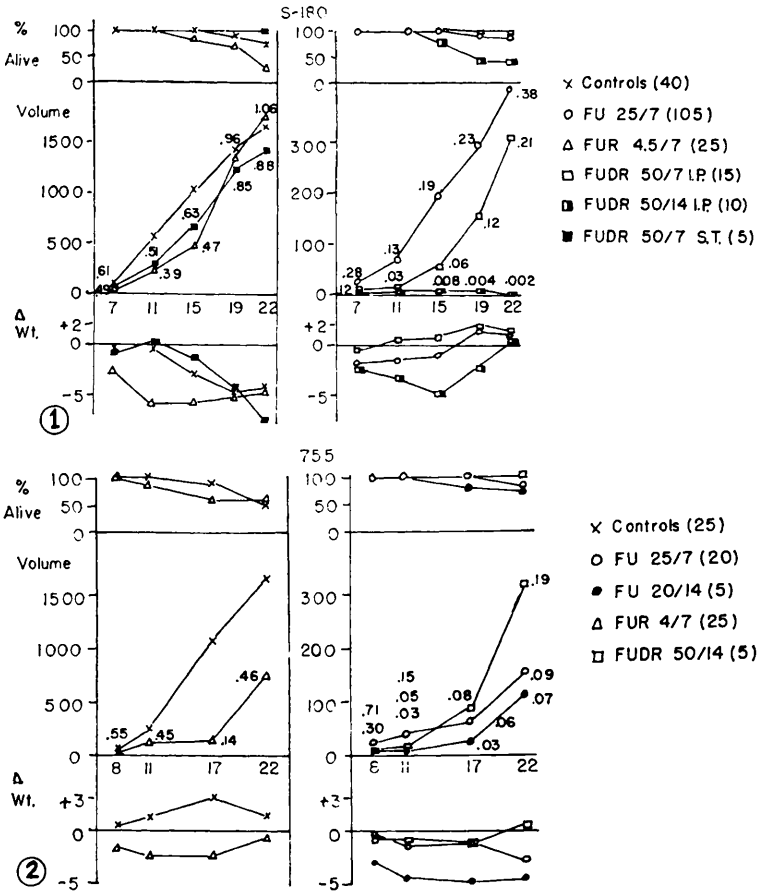


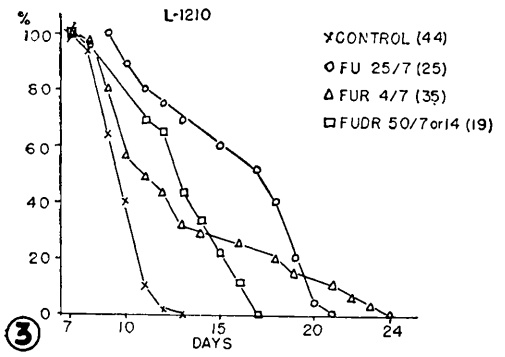
FIG. 1. Effect of fluorinated pyrimidines on growth of Sarcoma 180. Survival, tumor vol in mm³, and wt change in g plotted against days after transplantation. The dose is expressed as mg/kg/No. of days. Values in parenthesis are No. of mice in the experimental groups.

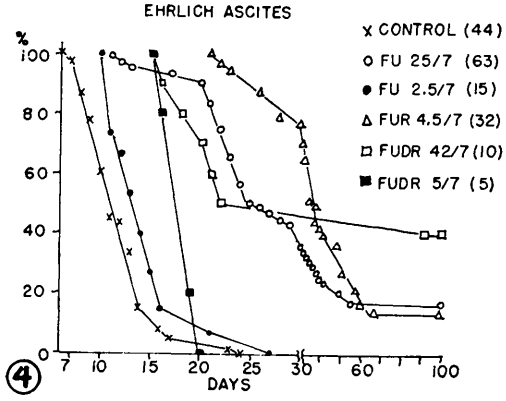
FIG. 2. Effect of fluorinated pyrimidines on growth of Adenocarcinoma 755. Survival, tumor vol in mm³, and wt change in g plotted against days after transplantation.

mained free of tumors to the 80th day when the experiment was terminated. The 3 remaining mice developed small tumors between the 28th and 41st day, and the tumors were removed for histological examination. The tissue structure of these specimens of retarded tumors resembled the microscopic appearance of Sarcoma 180. There were necrotic areas in the central parts of the tumors, but the peripheral zones showed all signs of active growth and mitotic activity. In one of the tumors removed on the 41st day enlarged cells were found, similar to those reported by Clarke *et al.*(10) in Sarcoma 180 tumors treated with 6-mercaptopurine. No such cells have been observed following treatment with 5-fluorouracil.

755 Mammary Adenocarcinoma. The effects of the drugs on this tumor are shown in Fig. 2. It will be noted that all exerted a considerable inhibition of tumor growth rate until the 17th day, but that the effect wore off by the 22nd day particularly with FUR and FUDR. It is clear that in this tumor 5-fluorouracil, when given at 25 mg/kg for 7 or 14 days is more effective than either fluorinated nucleoside.

L-1210 Leukemia. The per cent survival of mice bearing this tumor in ascites form is plotted against days after transplantation in Fig. 3. In this tumor, as in the 755, 5-fluorouracil is more effective than either of its nucleosides. A few mice treated with 5-fluorouridine survived longer than those treated





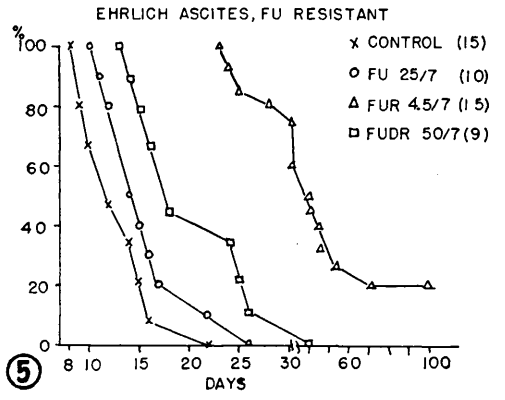


FIG. 3. Effect of fluorinated pyrimidines on survival of mice bearing L-1210 leukemia in ascites form. Per cent survival plotted against days after transplantation.

FIG. 4. Effect of fluorinated pyrimidines on survival of mice bearing Ehrlich ascites carcinoma. Per cent survival plotted against days after transplantation.

FIG. 5. Effect of fluorinated pyrimidines on survival of mice bearing a 5-fluorouracil-resistant line of Ehrlich ascites carcinoma. Per cent survival plotted against days after transplantation.

with 5-fluorouracil, but this is of questionable statistical significance. If the 50% survival

TABLE I. Novikoff Hepatoma.

Days after transplantation on which rats died		
Controls	5-Fluorouracil, 25 mg/kg/7 days	5-Fluorouridine, 10 mg/kg/7 days
8	11	21
8	12	22
9	12	30
9	13	100*
9	13	100*
10	15	100*
Avg 8.8	12.7	

* These rats were killed and found to be tumor-free at 100 days.

is noted, FU is considerably more effective than FUR.

Novikoff Hepatoma. The survival times of small groups of rats treated with FU and FUR are shown in Table I. Although a dose of 5 mg/kg/7 days of 5-fluorouridine produced considerable weight loss in mice, 10 mg/kg/7 days was well tolerated by rats, and at that dose the compound is a very effective inhibitor of this tumor. For comparison the lesser effect of 25 mg/kg/7 days of 5-fluorouracil is shown; this dose corresponds to 4 times the molar equivalent of FUR given in this experiment.

Ehrlich Ascites Carcinoma. The results of a series of survival time experiments are shown in Fig. 4. All 3 compounds were effective against this tumor only if given by intraperitoneal injection. It will be noted that 40% of the mice treated with 5-fluoro-2′-deoxyuridine survived for 100 days tumor-free whereas with 5-fluorouracil and 5-fluorouridine 18 and 15% respectively survived the experiment. Evidence that these mice were actually free of tumor cells was gained as follows. Sterile saline was injected intraperitoneally into 100-day survivors and withdrawn. Such lavage should be expected to pick up some tumor cells that may have been present. The washings were then injected intraperitoneally into recipient mice. None of these animals developed tumors over a 90-day period. Thus the treated animals were presumed to be cured of the tumor.

The comparison of these compounds at various doses is of interest. The most effective drug, as indicated by the number of surviving mice, is FUDR. This was given at

a dose of 42 mg/kg/7 days, which is approximately the equimolar dose of 25 mg/kg/7 days of FU. Under these conditions, FUDR is more effective than FU. 5-Fluorouridine, on the other hand, at a dose of 4.5 mg/kg/7 days is slightly more effective at prolonging the life of these mice than 25 mg/kg/7 days of 5-fluorouracil. The latter compound at 2.5 mg/kg/7 days, which is about equimolar to 4.5 mg/kg/7 days of FUR, is completely ineffective. The equivalent dose of FUDR, 5 mg/kg/7 days, has relatively little effect. Therefore we see that against the Ehrlich ascites tumor at optimal doses, 5-fluoro-2′-deoxyuridine is the most effective of the 3 drugs. However, 5-fluorouridine is by far the most efficacious on a mg/kg basis, since it is active at a much lower dose.

The results obtained with these 3 compounds in a 5-fluorouracil-resistant line of the Ehrlich ascites carcinoma(2) are shown in Fig. 5. Here the resistance to FU was almost complete, and there was a partial resistance to FUDR. On the other hand, FUR was just as active against the 5-fluorouracil-resistant line as the susceptible line, with 50% survival times of 40 days in each case.

Discussion. On the basis of biochemical knowledge it is reasonable to suppose that nucleosides of nucleic acid analogs might have altered biological and tumor-inhibitory properties. An example of this is the observation by Schindler and Welch that the riboside of 6-azauracil is active against cells of Sarcoma 180 grown in tissue culture, whereas 6-azauracil itself is not(11). On the other hand, Skipper *et al.*(12) found that 6-mercaptopurine and its riboside had approximately the same activities against Adenocarcinoma 755.

In the series of fluorinated pyrimidines it is of interest that in mice the toxicity by repeated doses of 5-fluorouridine is much greater than that of 5-fluorouracil, whereas the toxicity of 5-fluoro-2′-deoxyuridine is less than that of FU on an equimolar basis. The most striking conclusion which can be drawn from this work is the variation of response of these transplanted tumors to the 3 compounds. In Sarcoma 180 5-fluoro-2′-deoxyuridine is much more effective than 5-fluorouracil; 5-fluorouridine exerts no significant effect on this tumor. In contrast, in both Adenocarcinoma 755 and L-1210 leukemia, 5-fluorouracil is the most effective of the 3 although FUDR and FUR exert significant inhibitions. In the Ehrlich ascites carcinoma, both nucleosides are more active than the free pyrimidine analog, with FUR effective at a much lower dose than the others. In the rat, 5-fluorouridine exerts less toxicity than in the mouse, and hence a dose of this compound twice as great as can be given to mice was much more effective than FU in inhibiting the growth of the Novikoff hepatoma. Insufficient supplies of FUDR did not permit tests against rat tumors. Whether the diversity in responses of the tumors to these 3 compounds is a reflection of different biochemical mechanisms of tumor-inhibition produced by these drugs, or whether it indicates differences in drug metabolism, tissue distribution, or cell permeability are matters currently under investigation.

The lack of cross-resistance between 5-fluorouridine and 5-fluorouracil in the FU-resistant line of the Ehrlich ascites carcinoma has obvious clinical implications, and the result is reminiscent of the observation by Handschumacher that a 6-azauracil-resistant line of *S. faecalis* was inhibited by azauracil riboside(13). In contrast, Skipper *et al.*(12) found that in 6-mercaptopurine-resistant strains of Adenocarcinoma 755 and *S. faecalis*, 6-mercaptopurine riboside had no effect. Whether this difference in response reflects a basic difference between the inhibitory mechanism of analogs of purine and pyrimidine nucleosides is not known, and studies of mechanism of action of the series of fluorinated pyrimidines in the resistant tumor are under investigation.

In spite of the inconsistent results obtained with various transplanted tumors, the undoubted tumor-inhibitory activities of 5-fluorouridine and 5-fluoro-2′-deoxyuridine make it appear highly desirable to overcome the severe technological difficulties of producing larger quantities of these substances in order to evaluate them clinically in human cancer.

Summary. 1. Activity of 5-fluorouridine (FUR) and 5-fluoro-2′-deoxyuridine (FU-

DR) against several transplanted tumors has been compared with that of 5-fluorouracil (FU). 2. In Adenocarcinoma 755 and L-1210 leukemia FU is more effective than either nucleoside, although the latter have significant tumor-inhibitory activities. 3. In Sarcoma 180, FUDR is much more effective than FU; FUR has no significant activity. 4. In the Ehrlich ascites carcinoma, both nucleosides are more effective in prolonging life than is 5-fluorouracil, with FUR demonstrating activity at a low dose. In a 5-fluorouracil-resistant line of the Ehrlich ascites carcinoma, 5-fluorouridine is as active as in the parent strain. 5. In mice, the toxicity by repeated doses of FUR is much greater than that of FU; FUDR is less toxic than FU. In rats, the toxicity of FUR is much less pronounced than in mice, and the compound was very effective in inhibiting the growth of the Novikoff hepatoma.

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Reversibility of Venular Dilatation and Congestion in Diabetic Subjects Over a Period of Hours. (23778)

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The pathogenesis of the late diabetic sequelae, retinopathy and glomerulosclerosis, remains unknown. With ophthalmoscope (magnification 15 x) the initial visible change in the retina of diabetics consists of venous congestion (phlebopathy) and small sanguinolent dots (micro-aneurysms)(1,2). By using the stereoscopic microscope (magnification 100 x) on the bulbar conjunctiva, one observes in a great number of young diabetics a dilatation and congestion of the venules associated with exudation(3). A relationship has been found between the degree of retinal changes and the conjunctival alterations(4). Venular dilatation in the conjunctiva is apparently a reversible change which varies in degree when observed at weekly intervals (5).

This investigation was undertaken to determine (1) whether venular dilatation fluctuates over a period of hours and (2) if so, whether it can be correlated with blood sugar levels or with time after administration of insulin.

Material and methods. Fifteen diabetic subjects admitted to the Hospital Teaching Clinic for regulation were chosen for study. Other than diabetes and its complications the subjects were free from disease. None had ketoacidosis. They included 6 females and 9 males whose ages varied from 10 to 43 years. The duration of diabetes ranged from 6