Effect of Deoxyuridine Coadministration on Toxicity and Antitumor Activity of Fluorouracil and Floxuridine

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Abstract □ The addition of deoxyuridine (UDR) to fluorouracil (FU) or floxuridine (5-fluoro-2′-deoxyuridine) (FUDR) produced a substantial increase in their toxicity in BDF_1 mice. Antitumor assays using sarcoma 180 tumor-bearing mice showed a concomitant increase in tumor growth inhibition for the nucleoside–drug combination over identical doses of the single drug. However, no significant increase in antitumor activity with the combination treatment was demonstrated when equitoxic doses were given. Additional support for the therapeutic equality of the single and combination drug regimens was the similarity of the therapeutic indexes for each treatment regimen involving either fluorouracil or floxuridine. The results suggested that any therapeutic benefit achieved with the combination therapy could be duplicated with either fluorouracil or floxuridine at a higher dose.

Keyphrases □ Deoxyuridine—effect of coadministration on toxicity and antitumor activity of fluorouracil and floxuridine □ Fluorouracil—effect of deoxyuridine coadministration on toxicity and antitumor activity □ Floxuridine—effect of deoxyuridine coadministration on toxicity and antitumor activity □ Anticancer agents—effect of deoxyuridine coadministration on toxicity and antitumor activity of fluorouracil and floxuridine

Coadministration of deoxyuridine (UDR) with fluorouracil (FU) and floxuridine (5-fluoro-2′-deoxyuridine) (FUDR) was proposed by Windheuser and Jato (1) who observed a conversion of 5-fluorouracil to 5-fluoro-2′-deoxyuridine by human blood. Because this transformation reduces the steps needed to convert fluorouracil to the active form (2, 3), an enhancement of the antitumor activity of fluorouracil and floxuridine by deoxyribonucleosides was predicted. If this mechanism is operative in murine tumor therapy, the antitumor activity of fluorouracil or floxuridine should be increased in the presence of an excess of deoxyuridine. Preliminary experiments on transplanted mouse tumors, L-1210 leukemia and mammary adenocarcinoma 755, showed that deoxyuridine administered together with fluorouracil or floxuridine enhanced the antitumor activity of each drug over the same dose of each drug alone (4).

To understand the clinical significance of this enhancement, combinations of deoxyuridine with fluorouracil and floxuridine were examined in an animal tumor system (sarcoma 180) and compared to the single drugs using the parameters of antitumor activity and toxicity.

EXPERIMENTAL

Materials—*N*-Methylformamide[1], fluorouracil[2] (pH 9.0, solution), floxuridine[2] (powder), and deoxyuridine[3] (thymine and thymidine free) were used as received.

Animals—Female BDF_1 mice[4], 20–22 g, were maintained on a standard pellet diet and tap water *ad libitum*.

Toxicity Studies—Following a 7–10-day period of acclimatization before experimental use, the BDF_1 mice were randomized and divided into groups of 10, corresponding to the number of drug regimens to be tested plus a control group. Drugs were put into solution with sterile 0.9% NaCl 24 hr prior to the 1st day of injection. Each mouse received intraperitoneally 0.01 ml/g body weight of the drug regimen for 7 consecutive days; control animals received an equivalent volume of sterile 0.9% NaCl.

For the combination drug regimens, a constant amount of deoxyuridine (200 mg/kg/day) was coadministered with each dose of fluorouracil and floxuridine. This level of the nucleoside, an approximate fivefold excess of deoxyuridine over floxuridine, was used in previous *in vivo* studies (4). Average weights for each group were determined daily, and deaths were recorded throughout the 3-week observation period.

Implantation and Treatment of Sarcoma 180 Tumor—The sarcoma 180 tumor was maintained in the laboratory by subcutaneous transplant in female BDF_1 mice every 7 days. Aseptic conditions were maintained by using sterilized instruments and working under a hooded area. For tumor transplantation, the tumor was excised from the animal and placed in a petri dish containing cold saline. The tumor tissue was trimmed of necrotic and fibrous tissue and then minced with scissors into fragments; these fragments were implanted subcutaneously over the front shoulder using a 16-gauge trocar. Tumor fragments were tested for sterility by placing samples into two tubes containing thioglycollate broth[5] and checking for bacterial growth after 48 hr. If growth was found, the experiment was discarded.

The antitumor activities of fluorouracil, fluorouracil plus deoxyuridine, floxuridine, and floxuridine plus deoxyuridine were assayed in groups of 10 BDF_1 mice given implants on Day 0. Test mice were given daily intraperitoneal injections for 7 days with a volume of 0.01 ml/g body weight of the appropriate drug solution, starting 24 hr after implantation of the tumors, *i.e.*, Day 1. Control mice received 0.9% NaCl in an identical manner. *N*-Methylformamide (NSC-3051) was used as a positive control at a dose of 200 mg/kg/day for 7 days.

On Day 12 postimplantation, the mice were sacrificed and each tumor was excised and weighed. The mean tumor weight for each group was used to calculate the values for test/control (T/C). Percent inhibition of tumor growth was given by Eq. 1:

$$\text{percent inhibition} = 100(1 - T/C) \qquad \text{(Eq. 1)}$$

RESULTS AND DISCUSSION

Determination of LD_{10} and LD_{50}—In a previous study, Jato and Windheuser (4) suggested that the increased antitumor activity produced when deoxyuridine was combined with fluorouracil or floxuridine was associated with an increase in toxicity for the combination drug regimens. To examine the effects of the nucleoside on toxicity of the single drugs, a series of dosages of fluorouracil and floxuridine were coadministered with deoxyuridine in BDF_1 mice (10 mice/dose) and deaths were recorded over a 3-week period. A plot of log dose–percent survivors enabled the determination of LD_{10} and LD_{50} values for each combination regimen from their respective graphs (Fig. 1).

The LD_{10} values of 30 mg/kg/day for fluorouracil and 150 mg/

[1] Eastman Kodak Co., Rochester, N.Y.
[2] Hoffmann-La Roche, Nutley, N.J.
[3] Sigma Chemical Co., St. Louis, Mo.
[4] A. R. Schmidt, Madison, Wis.
[5] Difco Laboratories, Detroit, Mich.

Table I—Lethal Doses for Fluorouracil plus Deoxyuridine and Floxuridine plus Deoxyuridine[a]

Drug	LD_{10}[b]		LD_{50}[c]	
	mg/kg/day	mmoles/kg/day	mg/kg/day	mmoles/kg/day
Fluorouracil plus deoxyuridine	12.0	0.092	16.0	0.123
Floxuridine plus deoxyuridine	58.0	0.236	67.0	0.272

[a] Drugs were given intraperitoneally for 7 consecutive days to groups of 10 BDF_1 mice/dose; the mice were observed for 2 weeks after the injection period; 200 mg/kg/day of deoxyuridine was coadministered with each drug. [b] Lethal dose for 10% of the mice. [c] Lethal dose for 50% of the mice.

kg/day for floxuridine have been reported (5). Because these values were obtained from a 5-day injection schedule, it was necessary to adjust the reported values to a 7-day injection period using the method described by Freireich *et al.* (6) before these values could be compared with the LD_{10} values obtained for the combination regimens in this study. The adjusted LD_{10} values were 21.4 mg/kg/day for fluorouracil and 107 mg/kg/day for floxuridine. A comparison with the experimentally determined values (Table I), 12 mg/kg/day for fluorouracil plus deoxyuridine and 58 mg/kg/day for floxuridine plus deoxyuridine, showed that the inclusion of deoxyuridine increased the toxicity of both fluorouracil and floxuridine approximately 100%.

In terms of relative toxicity, floxuridine plus deoxyuridine was less toxic than fluorouracil plus deoxyuridine on a milligrams per killogram per day and molar basis (Table I). This finding indicated that the addition of deoxyuridine, while enhancing the toxicity of the single drugs, did not alter the toxicity relationship; *i.e.*, floxuridine is less toxic than fluorouracil, as has been reported clinically (7) and demonstrated in mice (8).

Antitumor Activity—A preliminary study was performed in which a group of sarcoma 180-tumor bearing mice was injected for 7 consecutive days with varying doses of the nucleoside to determine if deoxyuridine exerted any antitumor activity. After 12 days postimplantation, the tumors were removed and weighed individually. The results of this study showed that deoxyuridine, even when administered at a dose 2.5 times greater than the level used in the combination treatment regimens, exhibited no antitumor activity.

The effect of deoxyuridine on the tumor growth-inhibitory activity of fluorouracil and floxuridine was then ascertained. When using the parameter of mean tumor weight on Day 12 postimplantation, the results in Table II show an increased antitumor activity for the combination drug regimens administered at dosages identical to those of the single drug regimens. These results, utilizing BDF_1 mice bearing sarcoma 180 tumors, corroborated the findings reported earlier in mice bearing L-1210 or adenocarcinoma 755 (4)—*viz.*, deoxyuridine increased the antitumor activity of fluorouracil and floxuridine.

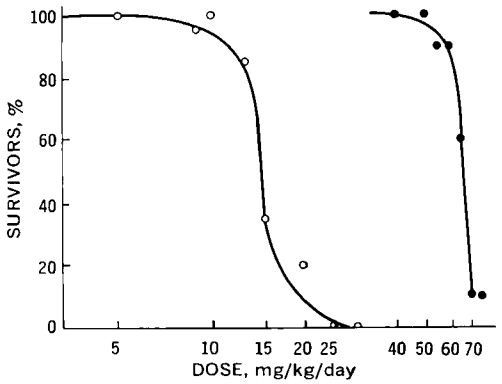

Figure 1—*Effect of deoxyuridine on lethality of fluorouracil* (○) *and floxuridine* (●). *Abscissa scale is logarithmic, but actual doses are indicated. Deoxyuridine was given at a dose of 200 mg/kg/day with each dose of the respective single drug.*

The loss in body weight that occurred with the higher dosages in the combination drug regimens suggested that the increased antitumor activity was achieved at the cost of a greater toxicity. The group that received 50 mg/kg/day of floxuridine had a net gain in weight of 1.2 g on Day 12 postimplantation, while the group that received 50 mg/kg/day of floxuridine in combination with 200 mg/kg/day of deoxyuridine experienced a net loss in weight of 2.0 g. A similar trend was produced by fluorouracil plus deoxyuridine at the highest dose (11 mg/kg/day).

Treatment of Sarcoma 180 with Equitoxic Levels—The preceding studies established that the addition of deoxyuridine to either fluorouracil or floxuridine resulted in an increase in the toxicity of the single drugs. Before a determination of increased antitumor efficacy could be ascribed to the combination drug regimens, it was necessary to compare the single drug regimen to the combination regimen at equitoxic levels rather than at identical dosages.

The results from such a study, using BDF_1 mice in which each drug regimen was given once daily for 7 consecutive days beginning 24 hr after implantation of sarcoma 180 tumor tissue subcutaneously, are shown in Table III. Each tumor was removed and weighed 12 days postimplantation; the mean tumor weight for each drug regimen was tabulated, and comparisons at equitoxic dosages were made between the single drug regimen and the corresponding combination drug regimen. The mean tumor weights of the mice that received deoxyuridine and fluorouracil were lower

Table II—Effect of Coadministered Deoxyuridine on Inhibition of Sarcoma 180 in BDF_1 Mice by Fluorouracil and Floxuridine[a]

Drug	Dose[b], mg/kg/day	Mean Body Weight Change[c], g	Tumors Evaluated	Mean Tumor Weight[d], g	T/C	Inhibition, %
Control (0.9% NaCl)	—	+0.8	41	6.16	—	—
Fluorouracil	5.5	+1.4	42	4.15	0.67	33
Fluorouracil plus deoxyuridine	5.5 / 200.0	+1.3	42	2.58	0.42	58
Fluorouracil	11.0	+1.4	42	4.27	0.69	31
Fluorouracil plus deoxyuridine	11.0 / 200.0	−0.1	41	0.73	0.12	88
Floxuridine	25.0	+1.2	42	2.85	0.46	54
Floxuridine plus deoxyuridine	25.0 / 200.0	+1.0	42	0.36	0.06	94
Floxuridine	50.0	+1.2	42	0.40	0.07	93
Floxuridine plus deoxyuridine	50.0 / 200.0	−2.0	40	0.03	0.01	99
N-Methylformamide	200.0	+0.8	42	1.13	0.18	82

[a] Tumor was implanted subcutaneously on Day 0. [b] Drugs were given intraperitoneally for 7 consecutive days starting on Day 1. [c] Between Days 1 and 12. [d] Tumors weighed on Day 12 postimplantation.

Table III—Effect of Equitoxic Levels of Single Drug and Combination Drug Regimens on Growth of Sarcoma 180 in BDF_1 Mice

Drug[a]	Dose Level[b]	Mean Tumor Weight[c], mg ± *SEM*		Mean Body Weight Change[d], g
Fluorouracil	3/4 LD_{10}	388 ± 74	NS[e]	+0.3
Fluorouracil plus deoxyuridine	3/4 LD_{10}	241 ± 25		−0.2
Fluorouracil	2/3 LD_{10}	467 ± 94	NS	+0.8
Fluorouracil plus deoxyuridine	2/3 LD_{10}	331 ± 71		+1.6
Fluorouracil	1/2 LD_{10}	582 ± 64	NS	+0.7
Fluorouracil plus deoxyuridine	1/2 LD_{10}	438 ± 66		+0.7
Floxuridine	2/3 LD_{10}	103 ± 17	NS	−0.6
Floxuridine plus deoxyuridine	2/3 LD_{10}	102 ± 18		−1.0
Floxuridine	1/2 LD_{10}	167 ± 41	NS	−0.1
Floxuridine plus deoxyuridine	1/2 LD_{10}	258 ± 38		+0.7
Floxuridine	1/4 LD_{10}	413 ± 76	NS	+0.9
Floxuridine plus deoxyuridine	1/4 LD_{10}	399 ± 84		0.0

[a] Drugs were given intraperitoneally for 7 consecutive days starting on Day 1. [b] Each group contained 15 BDF_1 mice; deoxyuridine was given at a dose of 200 mg/kg/day. [c] Tumors were weighed on Day 12 postimplantation. [d] Change in mouse body weight between Days 1 and 12. [e] NS = not significant ($p > 0.05$); significance value was determined by Student's *t* test.

than those that received only fluorouracil, but at no dosage level was the difference significant ($p > 0.05$). In the group that received floxuridine alone or in combination with deoxyuridine, there was much less of even an apparent difference in mean tumor weights between the two treatment regimens; again, no difference was significant.

No distinctive differences between the single drug and the combination drug regimens were evidenced in the net body weight changes over the 12-day experimental period. This finding suggested the equality of the regimens with regard to the toxicity produced at equal levels of antitumor activity.

The percent of tumor inhibition produced by each drug regimen was calculated from the mean tumor weight of each group and then plotted as a log dose–probit relationship (Figs. 2 and 3). By extrapolating from the appropriate figure, the effective dose that resulted in a 90% inhibition of tumor growth (ED_{90}) for each drug

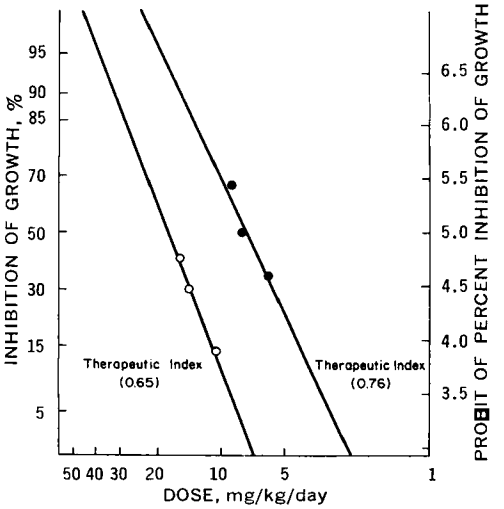

Figure 2—*Effect of fluorouracil and fluorouracil plus deoxyuridine on growth of sarcoma 180 in mice. Abscissa scale is logarithmic, but actual doses are indicated. Therapeutic index is represented by the ratio of LD_{10}/ED_{90}. Key: ○, fluorouracil; and ●, fluorouracil plus deoxyuridine (200 mg/kg/day).*

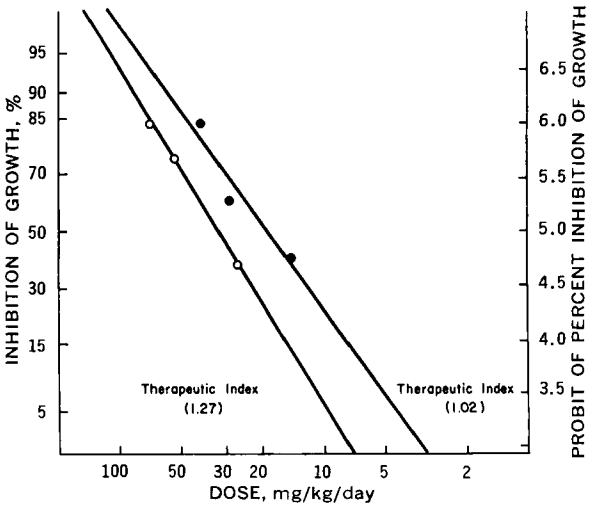

Figure 3—*Effect of floxuridine and floxuridine plus deoxyuridine on growth of sarcoma 180 in mice. Abscissa scale is logarithmic, but actual doses are indicated. Therapeutic index is represented by the ratio of LD_{10}/ED_{90}. Key: ○, floxuridine; and ●, floxuridine plus deoxyuridine (200 mg/kg/day).*

regimen was determined. By using these values, the therapeutic index, expressed by the ratio LD_{10}/ED_{90}, was calculated for each regimen.

The closeness of the calculated therapeutic indexes of fluorouracil and fluorouracil plus deoxyuridine, 0.65 and 0.76, respectively, indicated that the addition of deoxyuridine did not result in a therapeutic advantage for the combination drug regimen over the single drug regimen. This finding was reinforced by the therapeutic indexes of 1.27 for floxuridine and 1.02 for floxuridine plus deoxyuridine. Parenthetically, the superiority of both floxuridine and floxuridine plus deoxyuridine over the fluorouracil regimens was reflected by the larger therapeutic indexes, which denoted a greater margin of safety for the floxuridine regimens.

SUMMARY

When assayed against sarcoma 180, the combination of deoxyuridine (200 mg/kg/day) with fluorouracil or floxuridine produced an increase in antitumor activity over that produced by the identical dose of either single drug alone. Data from toxicity studies, however, revealed that the addition of deoxyuridine resulted in an increase in the toxicity of the combination regimens over the single drug regimens, as reflected primarily in mortality and secondarily in loss of body weight of BDF_1 mice.

When assayed at equitoxic dose levels, no significant increase ($p > 0.05$) in antitumor activity against sarcoma 180 was exhibited by either combination regimen over its respective single drug regimen. The addition of deoxyuridine to fluorouracil or floxuridine did not cause a notable change in either drug's therapeutic ratio (LD_{10}/ED_{90}). This finding suggested that any therapeutic benefit achieved with the combination drug regimen could be duplicated with either fluorouracil or floxuridine at a higher dose.

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Evaluation of a Dynamic Permeation Technique for Studying Drug–Macromolecule Interactions

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Abstract □ The applicability of a permeation rate technique to the determination of drug–macromolecule interactions was tested by measuring the extent of interaction of methylparaben with polyvinylpyrrolidone and polysorbate 80. Results were in agreement with literature data obtained by other techniques. The present method, although restricted to permeant molecules that diffuse readily through nonporous nylon membranes, is of potential value for investigations of drug binding by macromolecules not retained by porous dialysis membranes.

Keyphrases □ Permeation rate technique for determining drug–macromolecule interactions—tested using methylparaben with polyvinylpyrrolidone and polysorbate 80 □ Drug–macromolecule interactions—determination by permeation rate technique, compared to literature dialysis data

The phenomena of drug–macromolecule interactions have received wide attention in recent years in view of their relevance to drug absorption, transport, and overall availability. Dynamic dialysis[1] techniques have been successfully applied to the study of such interactions (1–3). These methods consist of the measurement of the rate of disappearance of a small molecule from a macromolecule-containing compartment under quasi-steady-state conditions. Their application purportedly offers some advantages (rapidity, economy, simplicity, *etc.*) over other time-honored techniques but is limited by the fact that porous dialysis membranes are pervious to many macromolecules of pharmaceutical interest.

Analogous techniques involving nonporous, lipid-like membranes have been proposed. Permeation rate methods (using nylon and dimethylpolysiloxane membranes) were applied to the study of complex formation between small molecules (4, 5). Nakano (6) investigated the interaction of chlorpromazine with several macromolecules, using a permeation rate method with dimethylpolysiloxane membranes, but his experimental system did not allow quantitative estimates of free and bound drug.

Nylon, whose permeability characteristics have been described (7), appeared to be an interesting membrane material for the study of drug–macromolecule interactions by a quasi-steady-state permeation rate technique. The purposes of this preliminary investigation were to evaluate the technique and to compare the results with literature data obtained by equilibrium dialysis.

EXPERIMENTAL

Materials—Methyl *p*-hydroxybenzoate[2] was recrystallized from methanol to a constant melting point of 127–128°. Polysorbate 80[3] and polyvinylpyrrolidone[4] were used as received. Nylon 6 (polycaprolactam) film from a single roll[5], in a labeled thickness of 0.5 mil (0.00127 cm), was used.

Apparatus—The permeation rate experiments were run with a specially designed cell (Fig. 1). The cell interior could be easily cleaned between experiments by removing the upper part (A) without disturbing the membrane. The nylon membrane was securely kept in place by a circular metal plate (C). Fluid tightness was ensured by two O-ring gaskets fitted in machined dies in the upper and lower part of the cell body (B). All parts in contact with the solution were either stainless steel or polytetrafluoroethylene[6] to avoid absorption of the diffusant by the cell material.

The approximate internal volume was 30 ml, and the diameter of the area available for diffusion was 6.0 cm. For use, the cell was placed in a jacketed beaker (internal height of 10.0 cm, internal diameter of 12.0 cm) connected to a thermostatted (30 ± 0.1°) water bath and circulator. Both the "internal" (cell) and "external" (beaker) solutions were stirred by synchronous motors[7]. One motor (60 rpm) was connected to the cell stirrer; the other (500 rpm) operated a magnetic stirrer.

To obtain reproducible results, newly cut membranes were soaked for at least 3 days in several changes of distilled water at 35°. Soaking at lower temperatures or for shorter times resulted in progressively decreasing permeation rates until an apparent stabilization occurred. This phenomenon resulted probably from insuf-

[1] The term dialysis is reserved here for a process involving diffusion through porous membranes, while permeation refers to diffusion through nonporous membranes.

[2] Carlo Erba, Milano, Italy.
[3] Tween 80, Atlas Europol SpA, Varese, Italy.
[4] Plasdone C, GAF Corp., New York, N.Y.
[5] Capran 77 C, Lot G/705283, Allied Chemical Corp., Morristown, N.J.
[6] Teflon, E.I. du Pont de Nemours & Co., Wilmington, Del.
[7] Crouzet S.A., Paris, France.