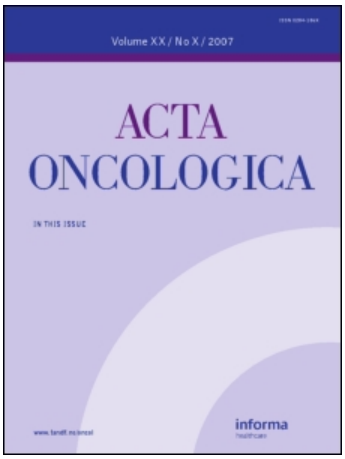

Acta Oncologica
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713690780

Cell Line Specific Radiosensitizing Effect of Zalcitabine (2',3'-dideoxycytidine)

Philippe A. Coucke [a]; Ye-Xiong Li [a]; Marie-Laure Copaceanu [a]; Nicolas Paschoud [a]; Eliane Cottin [a]; Mahmut Ozsahin [a]; René O. Mirimanoff [a]
[a] Laboratory of Radiobiology, Department of Radiation-Oncology, Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland

Online Publication Date: 01 January 1997

To cite this Article Coucke, Philippe A., Li, Ye-Xiong, Copaceanu, Marie-Laure, Paschoud, Nicolas, Cottin, Eliane, Ozsahin, Mahmut and Mirimanoff, René O.(1997)'Cell Line Specific Radiosensitizing Effect of Zalcitabine (2',3'-dideoxycytidine)',Acta Oncologica,36:2,199 — 205
To link to this Article: DOI: 10.3109/02841869709109230
URL: http://dx.doi.org/10.3109/02841869709109230

Cell Line Specific Radiosensitizing Effect of Zalcitabine (2′,3′-dideoxycytidine)

Philippe A. Coucke, Ye-Xiong Li, Marie-Laure Copaceanu, Nicolas Paschoud, Eliane Cottin, Mahmut Ozsahin, and René O. Mirimanoff

Laboratory of Radiobiology, Department of Radiation-Oncology, Centre Hospitalier Universitaire Vaudois, Lausanne Switzerland

Correspondence to: Dr Philippe A. Coucke, Laboratory of Radiobiology, Department of Radiation-Oncology, Centre Hospitalier Universitaire Vaudois Bugnon; 1011-Lausanne, Switzerland. Fax: +41.21.314.46.01; E-mail: pcoucke@chuv.hospvd.ch

Acta Oncologica Vol 36, No. 2, pp. 199–205, 1997

The potential of zalcitabine (ddC) to act as an ionizing radiation response modifier was tested on exponentially growing human cancer cells in vitro. Two human cell lines, WiDr (colon) and MCF-7 (breast) were exposed to ddC at 10 μM concentration for various lengths of time (18, 24, 48 and 72 h). On the WiDr cell line the dual effect of concentration and duration of exposure prior to irradiation was investigated. Experimental endpoints were clonogenicity and viability, as measured by colony formation assay (CFA) and MTT assay respectively. The impact on cell-cycle distribution prior to irradiation was assessed by flow cytometry using a double labeling technique (propidium iodide and bromodeoxyuridine pulse label). A significant reduction in surviving fraction and viability was observed for WiDr-cells irradiated after pre-exposure to 10 μM for 18, 48 and 72 h as compared to corresponding irradiated controls. At lower concentrations (1 and 5 μM), the radiosensitizing effect was only significant after a 72-h exposure (assessed by CFA). For MCF-7, ddC induced a significant modification of the dose response only with 24 and 48 h preincubation. However, the overall effect was less pronounced as compared to WiDr. Cell-cycle analysis showed accumulation in S-phase, 48 and 72 h after treatment with 10 μM ddC in the WiDr cells, with a progressive shift to late S-phase as shown by the biparametric analysis. The degree of radiosensitization is cell-line dependent with the most important sensitization observed on the most «radioresistant cell line», i.e., the cell line with the lowest alpha value and highest SF 2 (WiDr). For WiDr, radiosensitization by ddC depends on the duration of exposure and the concentration of the drug.

Received 29 February 1996
Accepted 10 December 1996

Nucleoside analogues (NA) were first investigated in the 1960s as potential biological response modifiers (BRM) of radiation response (1–11). Djordjevic (1) and Erikson (2) showed the capacity of bromodeoxyuridine and iododeoxyuridine to act as non-hypoxic radiosensitizers. The effect was thought to be related to the competition of halogenated nucleosides with native dTMP for subsequent phosphorylation and incorporation into DNA (7). Arabinofuranosyladenine (Ara-A) and arabinofuranosylcytosine (Ara-C) were shown by Iliakis to interact at the level of post-irradiation fixation of potentially lethal damage repair (5). Kim et al. demonstrated a negative feedback on de novo synthesis of DNA and RNA, redistribution of cells in G2 and subsequent radiosensitization by 6-thioguanine (8). However, not all NA will lead to radiosensitization. According to specific experimental conditions, some of them are potential radioprotectors (12).

More recently, drugs such as 2′-deoxy-2′(fluoromethylene) cytidine (FMdC) and 2′,2′-difluoro-2′deoxycytidine (dFdC) were described as BRM for radiation response in vitro (13). On the other hand, the triphosphate of ddC (ddCTP) has been shown to inhibit DNA-polymerase gamma and beta resulting in an impact at the level of mitochondrial DNA synthesis and DNA repair respectively (14). Therefore we decided to test this deoxycytidine derivative as a BRM, and to investigate the effect of exposure duration and concentration.

MATERIAL AND METHODS

Chemicals and drugs

Zalcitabine (ddC) was kindly provided by Bristol-Myers/Switzerland. DMSO, MTT and propidium iodide were purchased from Sigma Chemie (Buchs, Switzerland). Pepsin was purchased from Merck AG (Switzerland). MTT (3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyl tetra-

 ISSN 0284-186X

zolium bromide), a yellow tetrazolium salt, was dissolved at a concentration of 5 mg/ml in RPMI medium without phenol red, filtered and frozen for stockage.

Cell culture

Media and supplements were purchased from Gibco BRL (Basel, Switzerland). Fetal Calf Serum was obtained from Fakola AG (Basel, Switzerland).Two cell lines, WiDr and MCF-7, were purchased from the American Type Culture Collection (ATCC Rockville, MD) and grown on media as specified by ATCC. WiDr, a human primary adenocarcinoma of the rectosigmoid colon, was grown on minimum essential medium (MEM) with 0.85g/l NaHCO3, supplemented with 10% FCS, 1% non-essential amino acids, 2 mM L-glutamine, and 1% penicillin-streptomycin solution (pen-strep). The plating efficiency (PE) in these conditions was 99%. MCF-7, a human breast cancer cell line, was grown in MEM with 10% FCS, 0.5% pen-strep, 1% L-glutamine, 1 mM non-essential amino acids, 1 mM sodium pyruvate, and 10 μg/ml bovine insulin (90%). The PE for MCF-7 was approximately 40%.

For the MTT assay, the WiDr cells were grown on RPMI-1640 medium (without L-glutamine and phenol red), supplemented with 10% FCS and 1% pen-strep. Phenol red was suppressed in order to avoid the use of hydrochloric acid in the final solvent, which is known to alter the spectral properties of the formazan.

Irradiation/clonogenic assay

The cells were irradiated at room temperature with an Oris IBL 637 cesium source at a dose rate of 80.2 cGy/min (dose range 1 to 10 Gy with 1 Gy dose increment). Prior to irradiation exponentially growing cells were exposed to varying concentrations of ddC (1.5 and 10 μM) for WiDr, and to a single concentration of 10 μM for MCF-7. The exposure time ranged from 18 to 72 h. For the 48- and 72-h preirradiation exposure, the medium was changed every 24 h and supplemented with freshly prepared ddC. After trypsinization the cells were seeded in 100x20 mm Falcon Primaria culture dishes at adequate low density. Irradiation was done three hours after plating in order to allow cell attachment. For each irradiation dose four dishes were used for control and four dishes for ddC pretreated cells in parallel. An equivalent number of dishes were used to estimate baseline PE without radiation exposure. The clones (at least 50 surviving cells) were counted manually after fixation with 70% ethanol and staining with crystal violet.

The dose-modifying factor has been defined as the ratio of the surviving fraction of unexposed irradiated cells to the surviving fraction of ddC-treated and irradiated cells. These calculations were done at the 50% survival level (DMF-50) and at the 10% survival level (DMF-10).

Irradiation/MTT assay

The MTT assay was used because it is a rapid and semi-automatable technique. The original assay was modified according to Denizot & Lang (15) and Twentyman & Luscombe (16). The assay was performed with WiDr only. The cells were plated in a Falcon Primaria 96 well microplate at an appropriate concentration (4 300 cells/well in 200 μL medium). The assay was carried out in one half the area of the microtitre plate for the control and in the other half for the drug-exposed cells. The outer edges of the microtitre plate were filled with Earle's Bal. Salt (Gibco BRL) in order to avoid dehydration effects. Three hours after plating, the plates were irradiated at doses ranging from 1 to 10 Gy with a 1 Gy dose increment. To eliminate the apparent resistant tail at high dose, owing to the presence of doomed cells, some plates were irradiated at 15 Gy. The viability obtained at 15 Gy was subtracted from both treatment and control values at lower dose levels. After 72 h incubation the culture medium was removed, and the MTT procedure performed. The plates were read at a wavelength of 570 nm on a Spectra SLT (Tecan Ag., Hombrechtikon—Switzerland) plate recorder with the reference set at 690 nm.

Cell-cycle distribution/flow cytometry

The cells were prepared and exposed to ddC in exactly the same conditions as for the clonogenic assay. The cells were labeled with bromodeoxyuridine 30 min, then trypsinized and fixed in 70% ethanol. Cells were stained with propidium iodide for total DNA content. The cell suspension was stored in the dark at 4°C prior to the analysis. Biparametric flow cytometry yielded DNA content (red fluorescence; propidium iodine) and S-fraction (green fluorescence). The S-phase was arbitrarily divided into two equal sections to estimate accumulation in early and late S-phase compared to controls.

We analyzed 15 000 events, acquired on list mode using Multiplus (Phoenix Flow, San Diego, California).

Data analysis

Statistical analysis was carried out with the Statview software package on a Macintosh SE/30 computer. The curve fitting of the natural logarithm of SF (SF = surviving fraction) versus dose in Gy was done by a second degree polynomial regression analysis yielding the alpha and beta values for each experimental setting. The DMF-10 (dose-modifying factor at the 10% survival level) and DMF-50 (dose-modifying factor at the 50% survival level) were calculated using the fraction of the calculated SF of the control versus drug-exposed cells, after introducing the respective alpha and beta values obtained from each respective polynomial regression in the linear quadratic model.

Table 1

Effect on the surviving fraction at 2 Gy (SF-2): preradiation exposure to 10 μM ddC (72 h) of two cell lines from human origin (WiDr, colon cancer cell line; MCF-7, breast cancer cell line). The tabulated values are the mean values and corresponding standard errors calculated from experiments repeated at least thrice. The p-value (Student's t-test, two-sided) in the last column is issued from comparing all the experimental points (control vs. ddC) for the radiation reponse curve from 0 to 10

Time (h)	Control[c]		ddC—10 μM*		Δ[c] versus *
	Alpha	SF-2 (%)	Alpha	SF-2 (%)	p-value
WiDr					
18	0.24 ± 0.07	72.6 ± 3.8	0.32 ± 0.07	66.3 ± 2.7	<0.05
48	0.15 ± 0.04	73.9 ± 2.4	0.43 ± 0.04	64.5 ± 0.7	<0.05
72	0.11 ± 0.03	86.4 ± 2.1	0.42 ± 0.03	60.3 ± 2.4	<0.05
MCF-7					
24	0.9 ± 0.25	28.4 ± 3.3	0.76 ± 0.28	27.3 ± 3.6	<0.05
48	0.67 ± 0.19	31.2 ± 3.2	0.60 ± 0.3	29.8 ± 2.2	<0.05
72	0.60 ± 0.20	31.1 ± 2.5	0.31 ± 0.28	30.9 ± 5.8	0.4

The experiments were carried out simultaneously for control and drug-exposed cells for the whole range of doses in Gy (range 1–10 Gy), and each experimental set was repeated three times. Therefore, in order to compare survival data a two-sided paired *t*-test was used. A difference was considered statistically significant if a 0.05 p-value was reached. Standard errors were calculated and plotted on the corresponding figures.

RESULTS

Clonogenic assay

Treatment of WiDr and MCF-7 with up to 10 μM of ddC did not result in a significant change in the PE as compared to untreated controls. After 72 h exposure to 10 μM ddC, the surviving fraction at 2 Gy, calculated taking into account the corresponding baseline PE, was significantly reduced for pretreated WiDr (Table 1). For MCF-7, there was a difference at 24 and 48 h but this difference disappeared at 72 h. Taking the complete set of SF values (SF% from 1 to 10 Gy), the difference between pretreated cells (10 μM of ddC) and corresponding controls was statistically significant for WiDr at all exposure times, whereas for MCF-7 the values were only significantly different at 24- and 48-h exposure times (Table 1). However, the effect of 10 μM ddC was most pronounced for the WiDr-cells with the lowest alpha value and the highest SF 2, i.e., the most 'radioresistant' cell line (WiDr).

Subsequent experiments aimed at evaluating the importance of preincubation time and dose of ddC were therefore conducted only with WiDr. Reduction of the concentration of ddC from 10 to 1 μM at 72 h yielded a reduced radiosensitizing capacity. However, even at 1 μM concentration, we observed an increase in alpha and a reduced SF2 (see Table 2). At these lower concentrations of ddC (1 and 5 μM/48 h), a shorter exposure duration did not result in significant radiosensitization. The expected reduction in the radiosensitizing effect by lowering the concentration of ddC to 1 and 5 μM was counterbalanced by the prolongation of exposure to 72 h, resulting in significant sensitization even at low dose ddC (see Table 2). The importance of exposure duration was also illustrated at 10 μM ddC. The efficacy of 10 μM ddC as a radiosensitizer, especially in WiDr cells, is summarized in Table 3. Both DMF-10 and DMF-50 values are listed for both WiDr and MCF-7 cells at different exposure durations. The highest DMF value was obtained in WiDr cells exposed for 72 h to 10 μM ddC and reached 1.84.

Post-radiation exposure to very low dose ddC (0.5 μM) again yielded a small but statistically significant radiosensitizing effect (DMF-10 = 1.10 and DMF-50 = 1.23). Higher concentrations of ddC applied after irradiation induced a major reduction of PE that did not allow meaningful conclusions. As was the case with pre-irradiation exposure, the radiosensitization was mainly observed in the initial part of the dose-response curve.

MTT-assay

The viability test (MTT), as well as the flow cytometry, was performed on the cell line with the largest sensitization by ddC, i.e., WiDr. The viability of the cells exposed to up to 10 μM ddC was not significantly different from that of controls. For irradiated WiDr, the preirradiation exposure to 10 μM ddC consistently and significantly lowered the observed viability as compared to controls for all durations of exposure tested ($p < 0.05$). As observed in the clonogenic assays, the duration of exposure to ddC prior to irradiation significantly influenced the observed effect on viability with the most pronounced reduction of viability observed with a 72 h exposure to ddC.

Flow cytometry

The cell-cycle effects of ddC prior to irradiation were analyzed on WiDr, at the single concentration of 10 μM (24, 48 and 72 h exposure prior to irradiation). Biparamet-

Table 2

Impact of duration of exposure and concentration of ddC on the radiation response of exponentially growing WiDr in vitro. The tabulated values are the mean values and corresponding standard errors calculated from experiments repeated at least thrice. The p-value (Student's t-test, two-sided) in the last column is issued from comparing all the experimental points (control versus ddC) for the radiation reponse curve from 0 to 10 Gy

Time (h)	Control[c]		ddC*		$\Delta^{(c)\text{ versus }*}$
	Alpha	SF-2 (%)	Alpha	SF-2 (%)	p-value
ddC–10 μM					
18	0.24 ± 0.07	72.6 ± 3.8	0.32 ± 0.07	66.3 ± 2.7	<0.05
48	0.15 ± 0.04	73.9 ± 2.4	0.43 ± 0.04	64.5 ± 0.7	<0.05
72	0.11 ± 0.03	86.4 ± 2.1	0.42 ± 0.03	60.3 ± 2.4	<0.05
ddC–5 μM					
48	0.23 ± 0.06	67.7 ± 3.1	0.28 ± 0.08	68.6 ± 2.7	0.45
72	0.17 ± 0.08	75.9 ± 3.6	0.36 ± 0.09	69.4 ± 2	<0.05
ddC–1 μM					
72	0.17 ± 0.08	75.9 ± 3.6	0.29 ± 0.11	68.8 ± 6	<0.05

ric analysis (double staining with PI and BrdUrd) showed significant differences in cell-cycle distribution as compared to controls (Figure). At 24 h after subcultivation, there seemed to be a delayed progression through the cell cycle with significantly more control cells having progressed to late S and G2 + M as compared to ddC-treated cells. At 48 and 72 h, there was a significant accumulation of cells treated with ddC 10 mM in the S-phase. By dividing the green-labeled population midway between G0-G1 and G2 + M, an estimate was made of early S (ES) and late S (LS). At 48 h, both ES and LS were significantly increased by ddC, whereas at 72 h the overall S-phase increase was essentially due to LS accumulation.

DISCUSSION AND CONCLUSIONS

Cure from cancer undoubtedly requires local control. Radiation therapy aims at increasing local tumor control, but for a variety of tumors local recurrence remains a keystone in disease progression. To increase the efficacy of local radiotherapy, the intensity of the radiation treatment should be enhanced. For the radiation oncologist the Holy Grail is the quest for modalities that can enhance selectively the radiation effect on tumor cells while sparing normal tissues. Nucleoside analogues (NA) are interesting drugs because they should be preferentially incorporated in actively dividing tissues (6, 17). From 1960 on, different investigators have been testing NA as potential modifiers of radiation response. In vitro experiments yielded interesting results, especially for bromo- and iododeoxyuridine, ara-A and ara-C, fludarabine, chloro- and fluorodeoxycytidine and thioguanine (1–11, 18–21). Some of these NA are being actively investigated in clinical trials as radiosensitizers (22, 23).

Table 3

WiDr	72 h	48 h	18 h
DMF-10	1.33	1.26	1.05
DMF-50	1.84	1.57	1.2
MCF-7	72 h	48 h	24 h
DMF-10	1.04	1.04	1.06
DMF-50	1.02	0.95	1.05

Research in the field of NA has developed tremendously since their application in the context of AIDS and AIDS-related complex. Some of these NA were found to behave as potential modifiers of chemotherapy. Azydothymidine (AZT), acting on thymidine kinase and hence blocking the salvage pathway, has been shown to act synergistically with 5-fluorouracil (24). Recently, we published data on the effect of AZT on the radiation response of WiDr and HeLa cells in vitro. The expected sensitization did not occur. Azydothymidine seems to be able to reduce the radiation effect in vitro (12).

Zalcitabine (ddC) is particularly interesting because of its widespread clinical use in AIDS and AIDS-related complex (25–27). This deoxycytidine is known to have effects similar to, although less toxic than, AZT. It suppresses HIV-1 replication by inhibiting viral reverse transcriptase and/or by terminating viral DNA synthesis. It is, however, 10 times more potent than AZT, and does not have the same toxicity profile, and does not show cross-resistance; the reason why it has been extensively studied in monotherapy or in combination with AZT (25). It is known to reduce the activity of the DNA polymerase beta and inhibit the mitochondrial DNA polymerase gamma (14). The S-phase accumulation observed might be an illustration of this effect on DNA polymerase. Moreover, it has been shown that ddC is able to decrease the direct buffering of calcium by mitochondria (28). It is potentially interesting to investigate whether this alteration of Ca^{++} might result in a modification of Ca^{++} dependent endonuclease activity and related apoptosis.

On the other hand, halogenated derivatives of deoxycytidines (5-bromo-2'deoxycytidine and 5-chloro-2'deoxycytidine) have been shown to act as radiosensitizers and cytotoxic agents (9). Their mechanism of action is at the level of deoxycytidine kinase or cytidine deaminase and

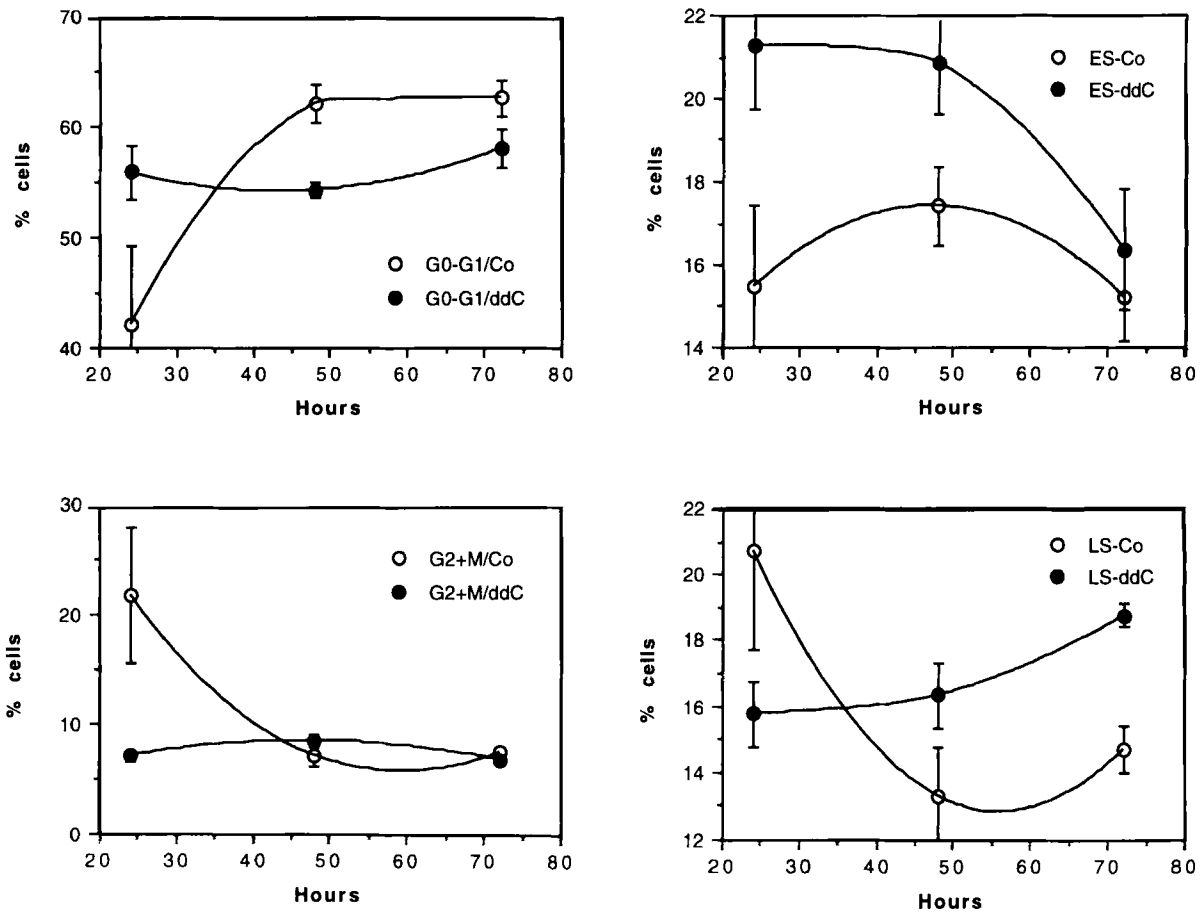

Figure. 1. Cell-cycle distribution of exponentially growing unirradiated WiDr exposed to 10 μM ddC (closed symbols) for 24, 48 and 72 h, compared to corresponding controls (open symbols). Medium and drug change was done every 24 h. Upper left: number of G0-G1 cells in % as a function of time to exposure in hours. Upper right: number of cells in early S-phase (ES). Lower left: number of cells in G2 + M. Lower right: number of cells in late S-phase (LS).

thymidine kinase (salvage pathway). The phosphorylated halogenated deoxyuridine will be incorporated as a thymidine analogue after a further two phosphorylations. These compounds are cytotoxic, and there is a concentration dependent decrease in cell survival. Their capacity to act as radiosensitizers is undoubtedly higher than that of ddC when one compares the obtained DMF values. Zalcitabine, as a deoxycytidine, has no cytotoxic effect per se at concentrations up to 10 μM in vitro. Nevertheless, a significant increase in radiation sensitivity was observed which seemed cell-line dependent. The dose modifying effect was most pronounced in the cell line with the lowest alpha value (WiDr). In this «resistant cell line», the observed effect increased with increasing concentration of ddC (range 1 to 10 μM) and prolonged exposure (range 18 to 72 h). The correlation between exposure time concentration and radiosensitization has already been observed for halogenated pyrimidines such as BrdUrd and Ird Urd (11, 29).

Cell synchronization, although observed in certain conditions does not in fact support the observed effect. The redistribution of cells in S-phase was not consistently found at all time points, nor in all cell lines. The increase in the S-phase fraction of the non-synchronized population of WiDr cells exposed for 48 h and 72 h to 10 μM ddC resulted in a significant radiosensitization. Biparametric analysis revealed progresssive accumulation of cells in the late S-phase especially at 72 h, although a significant global S-phase accumulation had already occurred at 48 h. The increase in radiation sensitivity and S-phase accumulation seems contradictory (30). It is as yet unclear whether the S-phase accumulation may be a result of direct interaction of ddC with thymidine kinase (TK) which is required in cell proliferation or by inhibition of DNA polymerase gamma (14, 31). On the other hand, the known inhibitory effect on DNA polymerase beta and hence the reduction of DNA repair may explain the marked change in the alpha component of the dose response.

Zalcitabine (ddC) applied to exponentially growing tumor cells acts as a radiosensitizer in vitro. The importance of the radiosensitization depends upon the cell line, and seems more pronounced in the cell line with the lowest

alpha value, in agreement with the results of Miller et al. (32). Using WiDr, a ddC-sensitive cell line, we demonstrated the importance of dose and timing of the drug. The effect appears on the initial slope of the most radioresistant cell line. The reduction of the shoulder width without a significant change in beta value (the dose-squared term) implies an increase in the single hit character of low LET radiation (20). More radiosensitive cell lines such as MCF-7 show less or no enhancement. However, a steep dose-response relationship will make small changes difficult to detect, especially considering the sensitivity of the methods used. The major advantage of ddC, however, is its potential to act as a radiosensitizer at low concentrations and especially at clinical-relevant dose levels, provided the exposure duration is long enough. At these concentrations there is no significant cytotoxic effect per se (no significant change in plating efficiency). This, of course, could potentially result in an interesting therapeutic index if there is no major bone marrow toxicity, which should be tested first on an animal model.

REFERENCES

1. Djordjevic B, Szybalski, W. Genetics of human cell lines. III. Incorporation of 5-bromo and 5-iododeoxyuridine into the deoxyribucleic acid of human cells and its effect on radiation sensitivity. J Exp Med 1960; 112: 509–31.
2. Erikson EL, Szybalski W. Molecular radiobiology of human cell lines. III. Radiation sensitizing properties of 5-iododeoxyuridine. Cancer Res 1963; 23: 122–30.
3. Grégoire V, Hunter N, Brock W, Milas L, Plunkett W, Hittelman WN. Fludarabine improves the therapeutic ratio of radiotherapy in mouse tumors after single-dose irradiation. Int J Radiat Oncol Biol Phys 1994; 30: 363–71.
4. Heimburger DK, Shewach DS, Lawrence TS. The effect of fluorodeoxyuridine on sublethal damage repair in human colon cancer cells. Int J Radiat Oncol Biol Phys 1991; 21: 983–7.
5. Iliakis G. Effects of beta-arabinofuranosyladenine on the growth and repair of potentially lethal damage in Ehrlich ascites tumor cells. Radiat Res 1980; 83: 537–52.
6. Iliakis G, Kurtzman S. Keynote address: application of non-hypoxic cell sensitizers in radiobiology and radiotherapy: rationale and future prospects. Int J Radiat Oncol Biol Phys 1989; 16: 1235–41.
7. Iliakis G, Kurtzman S, Pantelias G, Okayasu R. Mechanism of radiosensitization by halogenated pyrimidines: effect of BrdU on radiation induction of DNA and chromosome damage and its correlation with cell killing. Radiat Res 1989; 119: 286–304.
8. Kim JH, Alfieri AA, Kim SH, Hong SS. Radiosensitization of two murine fibrosarcomas with 6-thioguanine. Int J Radiat Oncol Biol Phys 1990; 18: 583–6.
9. Lawrence T, Davis MA. Selective radiosensitization and cytotoxicity of human melanoma cells using halogenated deoxycytidines and tetrahydrouridine. Int J Radiat Oncol Biol Phys 1989; 16: 1243–6.
10. Lawrence T, Heimburger DH, Shewach DS. The effects of leukovorin and dipyridamole on fluoropyrimidine-induced radiosensitization. Int J Radiat Oncol Biol Phys 1991; 30: 377–81.
11. Lawrence T, Davis MA, Maybaum J, Stetson PL, Ensminger WD. The dependence of halogenated pyrimidine incorporation and radiosensitization on the duration of drug exposure. Int J Radiat Oncol Biol Phys 1990; 18: 1393–8.
12. Copaceanu M-L, Coucke PA, Cottin E, Paschoud N, Mirimanoff R-O. Azidothymidine (AZT) as a potential modifier of radiation response in vitro. Acta Oncol 1995; 34: 213–8.
13. Perez LM, Greer S. Sensitization to x-rays by 5-chloro-2′deoxycytidine, co-administered with tetrahydro-uridine in several mammalian cell lines and studies of 2′-chloro derivatives. Int J Radiat Oncol Biol Phys 1986; 12: 1523–7.
14. Cherrington JM, Allen SJ, McKee BH, Chen MS. Kinetic analysis of the interaction between the diphosphate of (S)-1-(3-hydroxy-2-phosphonylmethoxypropyl) cytosine, ddCTP, AZTTP, and FIAUTP with human DNA polymerase beta and gamma. Biochem Pharmacol 1994; 48: 1986–8.
15. Denizot F, Lang R. Rapid colorimetric assay for cell growth and survival: Modifications to the tetrazolium dye procedure giving improved sensitivity and reliability. J Immunol Methods 1986; 89: 271–7.
16. Twentyman PR, Luscombe M. A study of some variables in a tetrazolium dye (MTT) based assay for cell growth and chemosensitivity. Br J Cancer 1987; 56: 279–85.
17. Kinsella T. Radiosensitization and cell kinetics: clinical implications for S-phase specific radiosensitizers. ASCO Educational Book 27th Annual Meeting 1991, pp 174–81.
18. Rockwell S, Grindey GB. Effect of 2′,2′-Difluorodeoxycytidine on the viability and radiosensitivity of EMT6 cells in vitro. Oncol Res 1992; 4: 151–5.
19. Sano K, Hoshino T, Hagai M. Radiosensitization of brain tumor cells with a thymidine analog (bromouridine). J Neurosurg 1968; 28: 530–8.
20. Shewach DS, Hahn T, Chang E, Hertel LW, Lawrence T. Metabolism of 2′,2′-Difluoro-2′-Deoxycytidine and radiation sensitization of human colon carcinoma cells. Cancer Res 1994; 54: 3218–23.
21. Snyder RD. Effect of 2′Deoxy-2′(fluoromethylene) cytidine on the ultraviolet and x-ray sensitivity of HeLa cells. Oncol Res 1994; 6: 177–82.
22. Kinsella TJ, Mitchell JB, Russo A, Aiken M, Morstyn G, Hsu SM, Rowland J, Glatstein E. Continuous intravenous infusions of bromodeoxyuridine as a clinical radiosensitizer. J Clin Oncol 1984; 2: 1144–50.
23. Wirtanen GW, Wiley AL, Vermund H, Stephenson JA, Ansfield FJ. Intra-arterial iododeoxyuridine infusion combined with irradiation. Am J Clin Oncol 1990; 13: 320–3.
24. Weber G, Nagai M, Prajda N, Nakamura H, Szekeres T, Olah E. AZT: a biochemical response modifier of methotrexate and 5-fluorouracil cytotoxicity in human ovarian and pancreatic carcinoma cells. Cancer Commun 1991; 3: 127–32.
25. Gustavson LE, Fukuda EK, Rubio FA, Dunton AW. A pilot study of the bioavailability and pharmacokinetics of 2′,3′-Dideoxycytidine in patients with AIDS or AIDS related complex. J AIDS 1990; 3: 28–31.
26. Lipsky JJ. Zalcitabine and didanosine. Lancet 1993; 341: 30–2.
27. Mitsuya KJ, Weinhold PA, Furman MH, St Clair S, Nusinoff-Lehrmann RC, Gallo D, Bolognesi DW, Barry and Broder S. Long-term inhibition of HTLV-III/LAV (human immunodeficiency virus) DNA synthesis and RNA expression in T-cells protected by 2′,3′-dideoxynucleosides in vitro. Proc Natl Acad Sci USA 1987; 84: 2033–7.
28. Werth JL, Zhou B, Nutter LM, Thayer SA. 2′,3′-Dideoxycytidine alters calcium buffering in cultured dorsal root ganglion neurons. Molec Pharmacol 1994; 45: 1119–4.

29. Phillips TL, Bodell WJ, Uhl V, Ross GY, Rasmussen J, Mitchell JB. Correlation of exposure time concentration and incorporation of IdUrd in V-79 cells with radiation response. Int J Radiat Oncol Biol Phys 1989; 16: 1251–5.
30. Sinclair WK, Morton RA. X-ray sensitivity during the cell generation cycle of cultured Chinese hamster cells. Radiat Res 1966; 29: 450–74.
31. Boothman DA, Davis TW, Sahijdak WM. Enhanced expression of thymidine kinase in human cells following ionizing radiation. Int J Radiat Oncol Biol Phys 1994; 30: 391–8.
32. Miller EM, Fowler JF, Kinsella TJ. Linear quadratic analysis of radiosensitization by halogenated pyrimidines. I. Radiosensitization of human colon cancer cells by iododeoxyuridine. II. Radiosensitization of human colon cancer cells by bromodeoxyuridine. Radiat Res 1992; 131: 81–97.