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Selective Protection by Anguidine of Normal *versus* Transformed Cells against 1-β-D-Arabinofuranosylcytosine and Adriamycin[1]

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ABSTRACT

Anguidine, a protein synthesis inhibitor, has been shown previously to induce a reversible arrest of cell progression through all phases of the mitotic cycle without inducing appreciable cell kill. This "frozen" cell cycle state provided protection of Chinese hamster ovary cells against the lethal effects of 1-β-D-arabinofuranosylcytosine, Adriamycin, hydroxyurea, 5-fluorouracil, and hyperthermia. We now report on the preferential induction of cytostasis by anguidine in normal WI-38 fibroblasts, occurring at one-tenth of the dosage required to inhibit the cycle progression of WI-38 VA13 cells, the SV40 transformant. Pretreatment with anguidine at a concentration producing effective inhibition of normal cell cycle traverse while permitting sustained proliferation of transformed cells resulted in almost complete protection of WI-38 normal cells against the growth-inhibitory effects of 1-β-D-arabinofuranosylcytosine and Adriamycin, without reducing the antiproliferative effects of these two agents against WI-38 VA13 transformed cells. Thus, this cytokinetic concept of preferential normal tissue protection should be explored *in vivo* to increase the therapeutic index of cancer chemotherapy.

INTRODUCTION

Human tumors do not necessarily proliferate faster than do their presumed normal tissue counterparts, thus providing a relative cytokinetic sanctuary for many antineoplastic agents (2, 3, 24). The observation of preferential reduction of normal cell cycle traverse rate by a variety of compounds should increase the therapeutic index of cancer therapy, because higher doses of cytotoxic drugs could be safely administered (12).

Three basic classes of agents have been investigated to date to promote selective interference with normal cell cycle progression. Cyclic adenosine 3':5'-monophosphate was shown by Pardee and James (19) to reduce hydroxyurea-induced killing of normal *versus* SV40-transformed baby hamster kidney cells. Rupniak and Paul (20) noted that the polyamine synthesis inhibitor methylglyoxal bis(guanylhydrazone) provided selective protection of 3T3 cells against the growth-inhibitory effects of hydroxyurea, via preferential block of G_1-S transition. Similarly, Sunkara *et al.* (22) demonstrated augmentation of ara-C[3]-induced cell kill in WI-38 SV40 cells by pretreatment with the ornithine decarboxylase inhibitor difluoromethylornithine, blocking normal WI-38 cells at the G_1-S interphase, while delaying the transformed cells in S. A similar cytokinetic property has been described for interferon, which has been reported by Greenberg and Mosny (11) to provide selective protection of normal *versus* leukemic colony-forming units in culture against ara-C. Finally, inhibitors of protein synthesis such as L-asparaginase (5), cycloheximide (4), and anguidine (23) can reduce the toxicity of S-phase-specific agents.

We have been interested in anguidine because of already available experience during Phase I and II trials, which were stimulated by the observation of remarkable activity against a mouse colon tumor (6, 7). While not active against a variety of human neoplasms, including gastrointestinal cancers, the side effects of anguidine were quite acceptable and predictable (17, 25).

Dosik *et al.* (8), studying the lethal and cytokinetic effects in cultured human colon cancer cells, found that anguidine blocked cell cycle progression at no cytotoxic expense. Further studies in Chinese hamster ovary cells showed that anguidine induced a concentration- and time-dependent universal cell cycle arrest, which reduced the toxicity of a variety of subsequently administered agents, including ara-C, Adriamycin, hyperthermia, 5-fluorouracil, and hydroxyurea (14, 23).

The present study was instigated to investigate whether anguidine could differentially arrest normal *versus* transformed cells and whether such differential arrest could provide selective host tissue protection against cytotoxic therapy. We found that anguidine suspended cycle progression of WI-38 cells at one-tenth of the concentration needed to arrest its SV40-transformed counterpart. This selective cell cycle arrest afforded preferential protection of WI-38 normal cells against ara-C and Adriamycin cytotoxicity.

MATERIALS AND METHODS

WI-38 cells and their SV40 transformants, WI-38 VA13 cells, were obtained from the American Type Culture Collection, Bethesda, Md., and were routinely grown in monolayer cultures in a 5% CO_2 humidified atmosphere at 37° in α-minimal essential medium (Grand Island Biological Co., Grand Island, N. Y.), supplemented with 10% heat-inactivated fetal calf serum (Irvine Scientific, Irvine, Calif.). Cells were harvested by treatment with 0.25% trypsin (Irvine Scientific) for 10 min at 37°. For these experiments, cell passages 24 through 30 were used.

For studies of differential cell cycle arrest, stathmokinetic experiments were carried out using DNA FCM after Colcemid treatment (9). Two hundred thousand cells were plated in 60-mm culture dishes and incubated for 48 hr to ensure exponential growth. Various concentrations of anguidine were added for an incubation period of 4 hr. After 2 washings with medium, fresh medium with Colcemid (0.1 μg/ml) was added, and dishes were further incubated for 24 hr. Cells were then harvested, washed, and fixed in cold 70% ethanol for cell cycle distribution analysis by DNA FCM using mithramycin and ethidium bromide as a highly DNA-specific dye pair (1). Stained cells were then

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[3] The abbreviations used are: ara-C, 1-β-D-arabinofuranosylcytosine; FCM, flow cytometry.

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analyzed in a Phywe ICP 11 flow cytometer (Ortho Instruments, Westwood, Mass.). Routinely, 20,000 to 30,000 cells were measured for each sample. Resulting DNA histograms were evaluated for cell cycle phase fractions using an algorithm developed by Johnston *et al.* (15).

All experiments were carried out in duplicate and repeated at least twice.

For studies of differential protection of normal *versus* transformed cells, 5×10^4 cells were plated in 35-mm culture dishes and incubated for 48 hr to ensure exponential growth. Anguidine at a concentration arresting normal but not transformed cells (1 μg/ml) was added for a 4-hr incubation period. After 2 washes with medium, fresh medium with either Adriamycin or ara-C was added. After a specified incubation period, the drug-containing medium was decanted, and dishes were washed 2 times with fresh medium. Cells were harvested at 24-hr intervals with 0.25% trypsin and counted using an electronic particle counter (Coulter Electronics, Inc., Hialeah, Fla.).

Anguidine, manufactured by Ben Venue Laboratories, was obtained from the Division of Cancer Treatment, National Cancer Institute, NIH, Bethesda, Md. Adriamycin was purchased from Adria Laboratories, Inc., Wilmington, Del. ara-C was purchased as Cytosar from Bristol Laboratories, Syracuse, N. Y. All drug solutions were prepared immediately prior to each treatment. Drugs were initially dissolved in 0.9% NaCl solution and further diluted in medium.

RESULTS

Differential Cell Cycle Arrest. As demonstrated by DNA FCM, untreated exponentially growing WI-38 VA13 cells had a 10% larger S-phase compartment size than did WI-38 cells (Table 1). Colcemid induced similar mitotic accumulations in WI-38 VA13 and WI-38 cells (75 to 80%). Addition of anguidine at increasing concentrations progressively interfered with mitotic accumulation, reaching almost pretreatment conditions at concentrations of 1.0 μg/ml in WI-38 and 10 μg/ml in WI-38 VA13 cells (Table 1; Chart 1). There was no further change in histogram distribution with further increase in drug concentration for both normal and transformed cell systems (not shown). Under treatment conditions effecting complete cell cycle arrest of WI-38 cells (1 μg/ml), WI-38 VA13 cell cycle progression was unaffected.

Selective Protection of Normal Cells. Studying the effect of treatment with anguidine (1 μg/ml) for 4 hr on WI-38 cell doubling, we noted a 2-day delay on subsequent normal WI-38 cell proliferation, whereas the transformed cell line remained unaffected (Chart 2). This differential arrest of normal cell growth provided selective protection against the antiproliferative effects of ara-C (500 μg/ml, 48 hr) and Adriamycin (10 μg/ml, 1 hr). Thus, in WI-38 VA13 cells, anguidine preincubation did not alter the steep decrease in cell number following both ara-C and Adriamycin treatment. In normal Wi-38 cells, Adriamycin killed at least 90% of the untreated cell population, while only 40% of cells were killed in the case of prior anguidine incubation. Four days after adriamycin treatment, both anguidine-treated and untreated cells showed recovery of exponential growth. A 48-hr exposure of WI-38 cells to ara-C almost completely prevented cell proliferation, whereas pretreatment with anguidine preserved exponential cell increase at a slightly reduced rate and with a delay of 2 to 3 days compared to control cells.

Table 1

Cytokinetic effects of anguidine on WI-38 and WI-38 VA13 cells

	G_0-G_1	S	G_2-M
Log growth control			
WI-38	65.3 ± 3.4[a]	27.6 ± 2.9	7.1 ± 1.4
WI-38 VA13	41.8 ± 3.5	37.2 ± 1.4	21.0 ± 2.0
Colcemid control			
WI-38	17.7 ± 2.4	4.6 ± 3.7	77.7 ± 2.9
WI-38 VA13	6.0 ± 0.8	17.8 ± 6.9	76.2 ± 7.1
ANG[b] (0.1 μg/ml) + COL			
WI-38	35.9 ± 5.1	5.6 ± 2.9	58.6 ± 2.9
WI-38 VA13	ND[c]	ND	ND
ANG (0.5 μg/ml) + COL			
WI-38	35.4 ± 2.5	26.8 ± 1.4	37.8 ± 1.1
WI-38 VA13	3.0 ± 0.7	19.8 ± 0.5	77.2 ± 0.2
ANG (1.0 μg/ml) + COL			
WI-38	44.3 ± 3.8	37.8 ± 3.6	17.9 ± 6.3
WI-38 VA13	12.4 ± 4.7	25.0 ± 6.6	62.6 ± 6.4
ANG (2.0 μg/ml) + COL			
WI-38	46.0 ± 2.5	40.1 ± 1.2	13.8 ± 3.6
WI-38 VA13	6.2 ± 4.4	28.2 ± 0.9	65.6 ± 5.3
ANG (5.0 μg/ml) + COL			
WI-38	46.6 ± 0.9	36.0 ± 1.9	17.4 ± 1.4
WI-38 VA13	10.6 ± 4.2	25.0 ± 10.5	64.4 ± 14.7
ANG (10 μg/ml) + COL			
WI-38	47.8 ± 1.2	36.4 ± 1.7	15.8 ± 0.8
WI-38 VA13	38.9 ± 5.4	40.9 ± 8.4	20.2 ± 3.1

[a] Mean ± S.E.
[b] ANG, anguidine; COL, Colcemid.
[c] ND, not done.

DISCUSSION

Anguidine inhibits protein synthesis by disaggregating polyribosomes (16), and inhibition of DNA synthesis is considered a secondary effect. The induction of cell cycle arrest may be related to the synthesis inhibition of proteins crucial for continuation in and transition between cell cycle phases. Compared to normal cell proliferation, the regulatory mechanisms of cell cycle phase transitions are weakened or absent in transformed cells (13, 17, 19), as supported by our finding that a 10-fold higher concentration of anguidine was required to block trans-

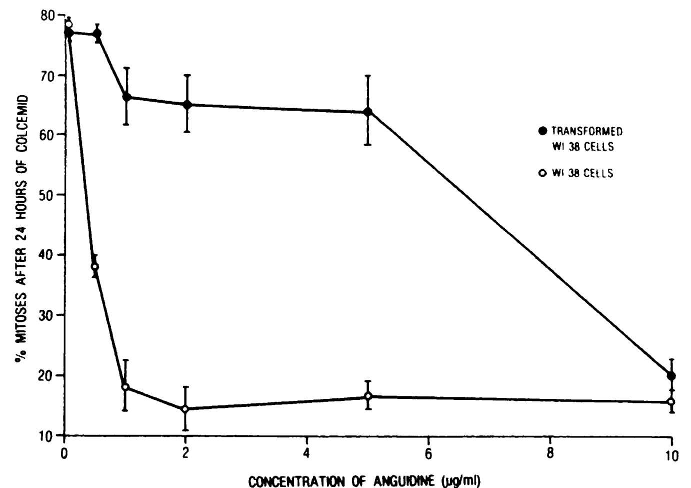

Chart 1. Colcemid-induced mitotic accumulation of VA13-transformed WI-38 and WI-38 cells after 4-hr pretreatment with anguidine at increasing concentrations. Anguidine prevents the mitotic accumulation of WI-38 cells at concentrations of ≥1 μg/ml. In contrast, a 10-fold higher concentration of anguidine is needed to prevent mitotic accumulation of the transformed WI-38 VA13 cells. There is a small subpopulation of transformed WI-38 cells that is relatively more sensitive to anguidine, as evidenced by a 15% drop in mitotic accumulation at anguidine (1 μg/ml). *Bars*, S.E.

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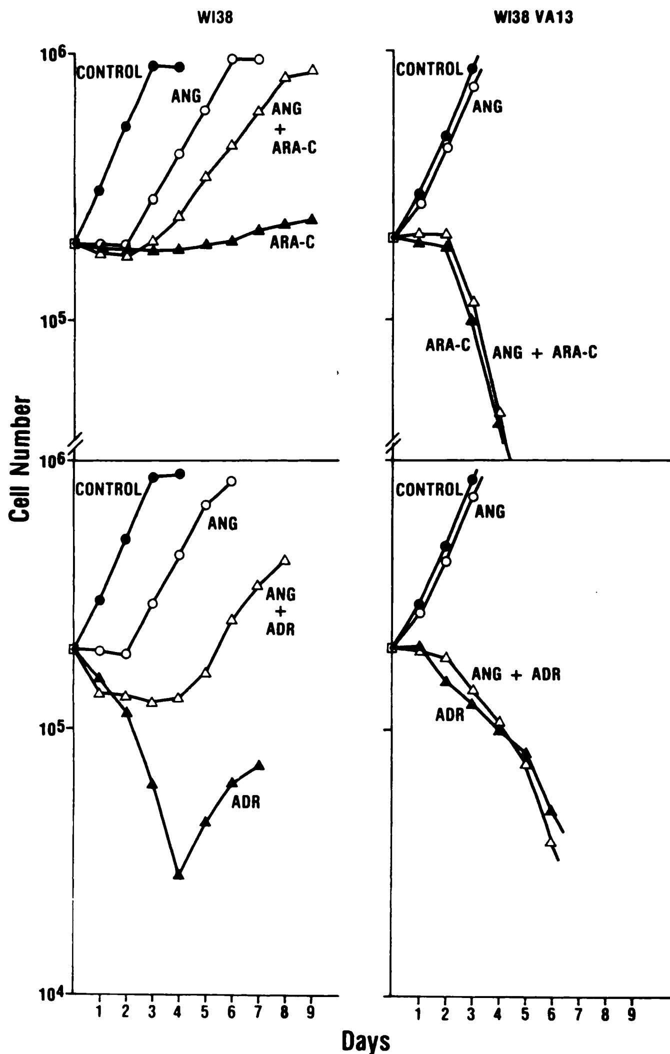

Chart 2. Cell growth curves of WI-38 (*left*) and WI-38 VA13 cells (*right*) following treatment with ara-C (500 μg/ml, 48 hr) (*top*) and Adriamycin (*ADR*; 10 μg/ml, 1 hr) (*bottom*). Note that preincubation with anguidine (*ANG*; 1 μg/ml) for 4 hr does not affect cell proliferation of WI-38 VA13 cells, whereas there is a 2-day growth delay in WI-38 cells. Anguidine pretreatment does not change the steep decline in cell number following both ara-C and Adriamycin treatment in the case of WI-38 VA13 cells. In contrast, normal WI-38 cells experience remarkable protection against Adriamycin and more so against ara-C. The average S.E. was ±22,000 cells for all experiments.

formed cells. We were able to exploit this differential antiproliferative sensitivity between normal and transformed cells to selectively protect normal cells against both an antimetabolite (ara-C) and a DNA-binding cytotoxic drug (Adriamycin). While such therapeutic advantage has been accomplished with other compounds (4, 5, 11, 19, 20, 22), the clinical side effects of anguidine are known and moderate (17, 25).

In preliminary studies with DNA FCM and tritiated thymidine autoradiography techniques in BALB/c × DBA/2 F_1 mice, we have demonstrated that 24 hr after i.v. injection of anguidine (3.3 mg/sq m) the marrow labeling index decreased by 90% without a change in DNA histogram configuration, a finding which is consistent with suspension of cycle progression. Thus, *in vivo* experimental and clinical studies are now under way to examine whether there is indeed a differential cytostatic effect of anguidine on normal *versus* malignant cells that can be exploited for normal tissue protection from cytotoxic drugs, thus permitting the safe delivery of substantially higher and more effective drug doses in a clinical setting.

REFERENCES

1. Barlogie, N., Göhde, W., Johnston, D. A., Smallwood, L., Schumann, J., Drewinko, B., and Freireich, E. J Determination of ploidy and proliferative characteristics of human solid tumors by pulse cytophotometry. Cancer Res., *38:* 3333–3339, 1978.
2. Baserga, R., Heneger, G. C., Kisielski, W. E., and Lisco, H. Uptake of tritiated thymidine by human tumors *in vivo*. Lab. Invest., *11:* 360–364, 1962.
3. Baserga, R., and Kisielski, W. E. Comparative study of the kinetics of cellular proliferation of normal and tumorous tissues with the use of tritiated thymidine. I—Dilution of the label and migration of labeled cells. J. Natl. Cancer Inst., *28:* 331–339, 1962.
4. Ben-Ishay, Z., and Farber, E. Protective effects of an inhibitor of protein synthesis, cycloheximide, on bone marrow damage induced by cytosine arabinoside or nitrogen mustard. Lab. Invest., *33:* 478–490, 1975.
5. Capizzi, R. L. Biochemical interactions between asparaginase (A'ase) and methotrexate (MTX) in leukemia cells. Proc. Am. Assoc. Cancer Res., *15:* 77, 1974.
6. Corbett, T. H., and Griswold, D. P. Treatment of colon adenocarcinoma in mice with anguidine (NSC-141537) and anguidine + 5-FU (NSC-19893). Proc. Am. Assoc. Cancer Res., *18:* 115, 1977.
7. Corbett, T. H., Griswold, D. P., Roberts, B. J., Peckham, J. C., and Schabel, F. M. Evaluation of single agents and combinations of chemotherapeutic agents in mouse colon adenocarcinomas. Cancer (Phila.), *40* (Suppl.): 2660–2680, 1976.
8. Dosik, G., Barlogie, B., Johnston, D. A., Murphy, W. K., and Drewinko, B. Lethal and cytokinetic effects of anguidine on a human colon cancer cell line. Cancer Res., *38:* 3304–3309, 1978.
9. Dosik, G. M., Barlogie, B., White, A., Göhde, W., and Drewinko, B. A rapid automated stathmokinetic method for determination of *in vitro* cell cycle transit times. Cell Tissue Kinet., *14:* 121–134, 1981.
10. Furth, J., and Cohen, S. Inhibition of mammalian DNA polymerase by the 5'-triphosphate of 1-β-D-arabinosyladenine. Cancer Res., *28:* 2061–2067, 1968.
11. Greenberg, P. L., and Mosny, S. A. Cytotoxic effects of interferon *in vitro* on granulocytic progenitor cells. Cancer Res., *37:* 1794–1799, 1977.
12. Hill, B. T., and Baserga, R. The cell cycle and its significance for cancer treatment. Cancer Treat. Rev., *2:* 159–175, 1975.
13. Holley, R. W. Control of growth of mammalian cells in cell culture. Nature (Lond.), *258:* 487–490, 1975.
14. Hromas, R., Barlogie, B., Drewinko, B., and Ho, D. H. W. Differential cell cycle arrest of normal versus transformed cells by the protein synthesis inhibitor anguidine. Proc. Am. Assoc. Cancer Res., *23:* 206, 1982.
15. Johnston, D. J., White, R. A., and Barlogie, B. Automatic processing and interpretation of DNA distribution: comparison of several techniques. Comput. Biomed. Res., *11:* 393–404, 1978.
16. Liao, L. L., Grollman, A. P., and Horwitz, S. B. Mechanism of action of 12, 13 epoxytrichothecene, anguidine, an inhibitor of protein synthesis. Biochim. Biophys. Acta, *454:* 273–284, 1976.
17. Murphy, W. K., Burgess, M. A., Valdivieso, M., Livingston, R. B., Bodey, G. P., and Freireich, E. J. Phase I clinical evaluation of anguidine. Cancer Treat. Rep., *62:* 1497–1502, 1978.
18. Pardee, A. B. A restriction point for control of normal animal cell proliferation. Proc. Natl. Acad. Sci. U. S. A., *71:* 1286–1290, 1974.
19. Pardee, A., and James, L. J. Selective killing of transformed baby hamster kidney (BHK) cells. Proc. Natl. Acad. Sci. U. S. A., *72:* 4994–4998, 1974.
20. Rupniak, H. T., and Paul, D. Selective killing of transformed cells by exploitation of their defective cell cycle control by polyamines. Cancer Res., *40:* 293–297, 1980.
21. Sartiano, G. P., Lynch, W. E., and Bullington, W. D. Mechanism of action of anthracycline and anti-tumor antibiotics, doxorubicin, daunomycin, and rubidazone: preferential inhibition of DNA polymerase alpha. J. Antibiot. (Tokyo), *32:* 1033–1045, 1979.
22. Sunkara, P., Fowler, S. and Nishioka, K. Selective killing of transformed cells in combination with inhibitors of polyamine biosynthesis and S-phase specific drugs. Cell Biol. Int. Rep., *5:* 991–997, 1982.
23. Teodori, L., Barlogie, B., Drewinko, B., Swartzendruber, D., and Mauro, F. Reduction of 1-β-D-arabinofuranosylcytosine and Adriamycin cytotoxicity following cell cycle arrest by anguidine. Cancer Res., *41:* 1263–1270, 1981.
24. Tubiana, M. The kinetics of tumor cell proliferation and radiotherapy. Br. J. Radiol., *44:* 325–347, 1971.
25. Yap, H. Y., Murphy, W. K., DiStefano, A., Blumenschein, G. R., and Bodey, G. P. Phase II study of anguidine in advanced breast cancer. Cancer Treat. Rep., *63:* 789–791, 1979.

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