Response of Exponentially Growing, Stationary-Phase, and Synchronized Cultured Human Colon Carcinoma Cells to Treatment with Nitrosourea Derivatives

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ABSTRACT

The lethal effects of two nitrosourea derivatives, 1,3-bis(2-chloroethyl)-1-nitrosourea and 4-[3-(2-chloroethyl)-3-nitrosoureido] cis-cyclohexane-carboxylic acid, on a continuous line of human colon carcinoma cells (LoVo cells) were investigated. The survival response of exponentially growing and stationary-phase LoVo cells to both drugs were of the threshold exponential type. Survival was identical whether drugs were dissolved in medium or in Hanks' balanced salt solution. In contrast to previous results obtained for human lymphoma cells, 1,3-bis(2-chloroethyl)-1-nitrosourea exerted a progressively greater killing effect on LoVo cells as the incubation time was prolonged, while 4-[3-(2-chloroethyl)-3-nitrosoureido] cis-cyclohexane-carboxylic acid, under similar circumstances presented decreased lethality in comparison to both 1,3-bis(2-chloroethyl)-1-nitrosourea and 4-[3-(2-chloroethyl)-3-nitrosoureido] cis-cyclohexyl)-1-nitrosourea (NSC 95441); PE, plating efficiency; LI, labeling index; 

INTRODUCTION

The use of nitrosourea derivatives in the systemic treatment of colorectal cancer has not been satisfactory because the elicited response rate was not greater than that obtained with 5-fluorouracil, the accepted standard of single-agent primary chemotherapy (11). To investigate the mechanisms of colonic cell killing by the agents and to provide some information on the possible reasons for their poor clinical performance, we treated an established human colon carcinoma cell line with 2 nitrosourea agents and analyzed the in vitro lethal effects by the colony-forming technique. Additionally, this investigation allowed us to compare present results with those previously published for an established human lymphoma cell line (14, 15) in order to detect tissue type-related differences. We utilized BCNU cl and cis-acid nitrosourea. BCNU was the first clinically useful nitrosourea derivative (32); it is readily soluble in alcohol, and its results in colorectal cancer approximated those obtained with MeCCNU, the best nitrosourea agent for this particular type of tumor (28). We did not use MeCCNU because this drug cannot be dissolved easily in a variety of solvents (15). cis-Acid is a new water-soluble nitrosourea (30) that was shown by Corbett et al. (13) to be very effective in mouse colon carcinoma.

MATERIALS AND METHODS

Cell Line. The cells used in this investigation were from a carcinembryonic antigen-producing colon carcinoma line (LoVo cells) established in 1972 (16). Cells are maintained as monolayer cultures in Ham's F-10 medium supplemented by 20% fetal calf serum, vitamins, glutamine, and antibiotics. LoVo cells generate glandular structures in vitro when grown as monolayers and in vivo when propagated as xenografts in nude mice (18). The generation time of exponentially growing cells is 29.3 hr, and the transit times through each stage of the cell cycle are: G1, 14.7 hr; S, 10.7 hr; and TG2+M, 4.8 hr. Single cells plated in fresh medium give rise to large colonies with a PE ranging from 35 to 70% (18).

Stationary-Phase Cultures. To establish the time sequence of the in vitro growth of LoVo cells (i.e., exponential and stationary phase), aliquots of 5 × 10^5 cells/dish were seeded into 60-mm Petri dishes containing 5 ml of growth medium and maintained without refeeding throughout the span of the experimental interval. Every 24 hr and for 21 days, the cells were harvested using previously described techniques (16) and were processed for cell counting, LI determinations, FCM studies, and colony formation. Cell counts were performed with the aid of an electronic particle counter (Model ZBI Coulter Counter, Coulter Electronics, Inc., Hialeah, Fla.). For LI determination, the cells were pulse labeled for 30 min with [3H]dThd (1 μCi/ml; specific activity, 3.0 Ci/mmol) before harvesting. Cytocentrifuge preparations were processed for radioautography using a 50% solution of liford K5 emulsion (Polysciences, Inc., Warrington, Pa.) in distilled water, exposed for 2 weeks, and developed in Kodak D19 (Eastman Kodak, Rochester, N. Y.). FCM studies were conducted on mithramycin-ethidium bro-mide-stained cells using a Phyne ICP 11 pulse cytophotometer (Phyne AG, Gottingen, Germany) (18). For PE determinations, aliquots of 200 cells/dish were dispensed into 60-mm Petri dishes.


Nitrosourea Effects on Colon Carcinoma

Kinetics of Cell Kill. Cell survival as a function of duration of treatment was investigated by exposing replicate cultures simultaneously to a single dose of drug. Petri dishes containing $5 \times 10^5$ cells in asynchronous exponential growth were incubated at 0 time with a single concentration of drug. Replicate cultures were harvested at regular intervals and processed for colony formation assays as described above. PE controls were obtained at regular intervals. To demonstrate the stability of the lethal activity of the nitrosourea, a separate control system was instituted. This consisted of dishes also containing $5 \times 10^5$ cells which were kept growing without adding drug throughout the experiment. Every time a time point dish was harvested, its supernatant medium was transferred to the control dishes after discarding their drug-free medium. The latter was now incubated for 1 hr at $37^\circ$, and survival was assessed as usual.

Recovery from Potentially Lethal Damage. To investigate recovery from potentially lethal damage, we used the method described by Little (27). Both exponentially growing and stationary-phase cells ($5 \times 10^5$ cells/dish) were treated with the nitrosourea agents (dissolved in medium or in HBSS) for 1 hr at $37^\circ$, washed thoroughly with HBSS, and reincubated for 8 and 24 hr with spent medium before harvesting for colony formation. Spent medium was the cell-free supernatant obtained from 2-week-old stock cultures. The survival of these cells was compared to that of cells harvested immediately after drug exposure was terminated.

Recovery from Sublethal Damage. To investigate recovery from drug-induced sublethal damage, cells were exposed to fractionated doses of the integral dose. Integral dose was defined as the product of the concentration times the time of incubation ($C \times t$) (5, 15, 23). Both stationary-phase and exponentially growing cells received the total integral dose in 2 equal separate exposures spaced in time, and their survival was compared to that resulting from cells receiving the total dose at one time. Controls obtained at regular intervals consisted of cells receiving: (a) one-half the integral dose; (b) the total integral dose; and (c) the total integral dose in which only the time parameter was changed.


cell synchrony was maintained in $G_1$, with 80% of the cells accumulated in that stage, but rapidly decreased when cells reached $G_2$. Twenty-four hr after release from the dThd block, the compartment distribution was similar to that observed for asynchronous cells.

Drugs. BCNU was obtained from the Cancer Chemotherapy Evaluation Branch, Division of Cancer Treatment, National Cancer Institute. It was received as a dry powder manufactured in injectable form by Ben Venue Laboratories (Bedford, Ohio) and was easily dissolved in pure ethanol before being admixed with growth medium or in HBSS. cis-Acid was obtained in crystalline form from the Kettering-Meyer Laboratory through the courtesy of Dr. J. A. Montgomery and was easily dissolved in 0.9% NaCl solution. For both agents, serial drug concentrations were prepared in growth medium or in HBSS. In all experiments, drug solutions were freshly prepared before treating the cells, and the pH was adjusted, if necessary, to 7.2.

Dose-Response Survival of Asynchronous Exponentially Growing Populations. Stock cultures were harvested by previously reported standard procedures (16) and counted with the aid of a Coulter Counter Model ZBI electronic particle counter. Cell suspension aliquots were seeded into 60-mm Petri dishes so that 50 to 100 colonies were accumulated in that stage, but rapidly decreased when cells reached $G_2$. Twenty-four hr after release from the dThd block, the compartment distribution was similar to that observed for asynchronous cells.


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Dose-Response Survival of Asynchronous Exponentially Growing Populations. Stock cultures were harvested by previously reported standard procedures (16) and counted with the aid of a Coulter Counter Model ZBI electronic particle counter. Cell suspension aliquots were seeded into 60-mm Petri dishes so that 50 to 100 colonies would appear after 21 days of incubation in a 5% CO$_2$ humidified atmosphere at 37$^\circ$. The drug was decanted, and the cells were washed twice in HBSS, harvested as a monolayer harvest. Known aliquots of the cell suspension were dispensed into 60-mm Petri dishes so that 50 to 100 colonies would appear after 21 days of incubation in a 5% CO$_2$ humidified atmosphere at 37$^\circ$. The colonies were stained with 2% crystal violet in 95% ethanol, and the colonies were counted. Because neither nitrosourea derivative induced cell detachment, the decanted supernatant medium and the washings were not combined with the monolayer harvest. Known aliquots of the cell suspension were dispensed into 60-mm Petri dishes so that 50 to 100 colonies would appear after 21 days of incubation in a 5% CO$_2$ humidified atmosphere at 37$^\circ$. The colonies were stained with 2% crystal violet in 95% ethanol. Viability was defined as the ability of single cells to give rise to a colony of $\geq 50$ cells. In each experiment, the PE of at least 6 control cultures was assessed simultaneously. Control cultures consisted of cells treated in exactly the same manner as the test cells but without receiving drug. The survival fractions for the different drug concentrations were normalized with respect to the individual controls for each experiment. All experiments were repeated at least twice with triplicate samples for each drug concentration.

Dose-dependent Survival of Stationary-Phase Cultures. Cells were seeded in 60-mm Petri dishes ($5 \times 10^5$ cells/dish) and maintained at 37$^\circ$ for 8 days without refeeding (see “Results”). The medium was decanted, and increasing drug solutions (in medium or in HBSS) were dispensed to the cells for 1 hr at 37$^\circ$. The drug was decanted, and the cells were harvested and processed for colony formation. Controls were cells in stationary phase that did not receive drug treatment.

RESULTS

Growth Kinetics. LoVo cells seeded at a density of 2.15 $\times 10^4$ cells/sq cm ($5 \times 10^5$ cells/dish) and maintained without refeeding demonstrated a period of exponential growth (doubling time, 37 hr) that lasted for about 4 days (Chart 1). After about 2 doublings, cells entered stationary phase as defined by no increments in cell number. The plateau segment of the growth curve lasted for about 4 days after which time cell death increased as reflected by a decrease in cell numbers with a halving time of 103 hr. The LI declined from a value of 33% during exponential growth to a value of 1% just at the time cultures entered stationary phase. However, simultaneously FCM-determined DNA-dependent compartment distributions revealed that about 15% of the cell population had an S-phase DNA content and that about 16% of the cells had a G$_2$-phase DNA content. No significant variations in PE as a function of

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stage growth (exponential, stationary, or decline) were noted. The fluctuations in PE were similar to those documented for experiments conducted at different times and with different batches of stock cells. Stationary phase could not be reverted to exponential growth by: (a) replacement of supernatant with fresh medium; (b) brief incubation with trypsin and reincubation with the same supernatant; and (c) harvesting and transferring the entire cell population to new dishes containing fresh medium. Only when cells were replated in fresh medium at a density lower than $6.45 \times 10^5$ cells/sq cm ($<1.5 \times 10^6$ cells/dish) was logarithmic growth resumed.

**Dose-dependent Survival.** The survival curves of exponentially growing LoVo cells exposed to increasing concentrations of both nitrosoureas (Chart 2) were of the threshold exponential type (BCNU: $D_0 = 12 \mu g/ml$, 1 hr; $D_0 = 7 \mu g/ml$, 1 hr. cis-Acid: $D_0 = 21 \mu g/ml$, 1 hr; $D_0 = 13 \mu g/ml$, 1 hr). Survival was identical for cells treated with drugs dissolved in HBSS or in medium. The survival of LoVo cells treated with BCNU was significantly reduced with respect to that previously noted for human lymphoma cells (14, 15). Both the sensitivity of the critical target [as quantified by the $D_0(17)$] and the capacity to absorb damage (reflected by the $D_0$) were considerably decreased for the colon cells. cis-Acid was also more effective on the colon cells accounted by an increased sensitivity of the critical target ($D_0$).

No difference in survival of LoVo cells treated in the stationary phase of growth with both BCNU and cis-acid was noted with respect to results obtained for cells treated in the phase of exponential growth. Again, this result was observed independently of whether the agents were dissolved in medium or in HBSS.

**Kinetics of Cell Kill.** Cell survival as a function of duration of treatment was investigated by exposing cells to a single concentration of each nitrosourea derivative for increasing periods of time. The biological stability of the drug was monitored by exposing control cells for 1 hr to the drug-containing supernatants obtained at regular intervals from the treated cells and comparing their survival to that obtained for the cell exposed for 1 hr to freshly prepared drug.

BCNU, at a concentration of $15 \mu g/ml$, exerted a progressively greater killing effect as the incubation time was prolonged (22% survival after 12 hr treatment). After 12 hr, the rate of cell killing declined, and survival was about 16% after 48 hr of continuous treatment (Chart 3). This result differs from that previously observed for cultured human lymphoma cells (15) in which after 4 hr a concentration of $25 \mu g/ml$ could not further increase the 55% killing effect achieved at that time. BCNU was relatively stable in solution; after 24 hr, the supernatant still elicited 70 to 85% of the killing effect of freshly prepared drug.

Cell kill by cis-acid followed a stepwise pattern (Chart 3).
Survival decreased rapidly for the first 4 hr; the rate declined for the next 24 hr, increased again after 30 hr, and declined once more after 36 hr. Survival of LoVo cells exposed to 25 µg/ml for 48 hr was 21%. Again, this result differs from that observed for lymphoma cells in which a concentration of 5 µg/ml produced a 5-log kill after only 4 hr (14). Furthermore, cis-acid in the supernatant of lymphoma cells was almost as effective after 24 hr as freshly prepared drug, while the lethal effect of the supernatant of LoVo cells decreased to 50% of freshly prepared drug after only 12 hr.

Recovery from Potentially Lethal Damage. Recovery from potentially lethal damage was investigated by maintaining treated LoVo cells in spent medium for 8 and 24 hr before harvesting for colony formation and comparing the survival curve to that of cells harvested immediately after drug exposure. Survival curves of cells harvested 8 and 24 hr after treatment were similar to those elicited for cells processed immediately after treatment (Chart 4). Again, this effect was identical independently of whether cells had been exposed to drug in HBSS or in medium. Thus, by using the method described by Little, we showed that LoVo cells do not have the capacity to repair potentially lethal damage.

Recovery from Sublethal Damage. LoVo cells in the exponential and stationary phases of growth were able to recover from sublethal damage induced by both nitrosourea derivatives (Chart 5). In fact, the response to a second half of the integral dose was not different from that to one-half dose alone.

Cell Cycle Stage-dependent Survival. No significant increase in cell killing as a function of position in the cycle was noted for treatment with both nitrosoureas (Chart 6). The difference between the highest and the lowest killing points (early G1 and early S, respectively) for BCNU was at most 3-fold. Less than a 2-fold difference was noted for cells treated with cis-acid. The corresponding dose-response survival curves obtained for each agent at different stages of the cycle were largely similar.

DISCUSSION

Nitrosourea derivatives are antitumor compounds that have been extensively investigated preclinically and clinically. They offer novel chemical structures, superior activity in experimental tumor systems, and unique modes of antitumor action (12, 37). All nitrosourea derivatives have qualitatively similar spectra of antitumor activities (9, 10), clinical toxicities (31), metabolic disposition (21), and degradation (34), and they appear to act through similar decomposition products (34, 38). Yet, important physicochemical differences (39) and different degrees of clinical effectiveness (22) and toxicity (31, 33) have been recorded. In previous studies on the effect of nitrosoureas on human lymphoma cells (14, 15), we documented quantitative and qualitative similarities among the various derivatives, but we also noted important biological differences which suggested that the killing modality of each of these agents may be different. We proposed that these differences may result from the
selective attack by each nitrosourea derivative on distinct target molecules or biochemical pathways. In addition, we elicited responses on the human cells which differed quantitatively and qualitatively from those reported by others for rodent cell lines, and we concluded that these were species-related differences. In this report, we demonstrate that differences in activity may not only be a function of the particular nitrosourea derivative but are also strongly related to the target cells used, even when the cells belong to the same species. In fact, we conclude that tissue type-dependent differences may be greater than those related to species origin.

Exponentially growing LoVo cells exposed to increasing concentrations of both BCNU and cis-acid for 1 hr at 37° revealed threshold exponential survival curves. Qualitatively, these curves were similar to those noted for other mammalian cells including our previously studied human lymphoma line (14, 15). However, LoVo cells proved significantly more sensitive to BCNU than the human lymphoma line with a killing effect approximating that described by other investigators for rodent cell lines (4, 8, 20, 36, 40). For both nitrosoureas, the greatest change was noted for the slope of the survival curve i.e., the segment that purportedly quantifies the sensitivity of the target molecule (17).

There was no difference in survival of LoVo cells treated in stationary phase with respect to results obtained for cells in exponential growth. Our data differ from the results reported by Tobey and Crissman (36) for CHO cells (about 5- to 10-fold difference in cell kill), by Bhuyan et al. (7) for L1210 leukemia (about a 3-fold increase in cell kill), and by Barranco et al. (5) for CHO cells (about 1000-fold increased kill) but are similar to the findings of Hageman et al. (19) for mouse plasmacytoma cells, of Thatcher and Walker (35) for hamster embryo cells, and of Hahn et al. (20) for HA1 cells.

These differences in results can be attributed, in part, to species-related biological changes. Another possibility resides in the fact that the stationary-phase cultures used by all investigators differ significantly in their cell cycle compartment distribution (7). Both Bhuyan and Barranco, using uptake of [3H]dThd, showed a decreased proportion of S-phase cells in plateau cultures (79 to 45% for L1210 cells and 62 to 4% for CHO cells). Bhuyan further documented this reduction in S-phase cells by FCM, although he also showed an increased proportion of cells in G2 + M. Barranco et al. (6) showed that, in their unfed plateau cultures, most cells were blocked in G1-like phase after 90 to 120 hr, although 1 to 4% of the population continued to incorporate [3H]dThd. Upon suspension in fresh medium, these plateau cells took almost 5 times longer to enter S phase than did true G1 cells synchronized by mitotic selection. This was followed by a parasychronous wave of cell division commencing after movement through S phase. That these G1-like plateau cells were not identical to true G1 cells was demonstrated by the fact that the plateau cells were 30 to 40 times more sensitive to bleomycin than were G1 cells (6) and that, while BCNU had not been particularly more sensitive for CHO cells in G1 phase (4), BCNU, MeCCNU, and 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (NSC 79037) were more effective (up to 1000-fold) against the plateau-phase cultures (5, 6).

For LoVo cells in plateau, the cell cycle stage compartment distribution was similar to that observed by Bhuyan et al.; i.e., a considerable proportion of cells present S phase (15%) and G2 + M (16%) DNA content, although the LI was only 1%. These results indicate that mammalian cells in the stationary phase of growth do not necessarily accumulate in G2 phase and that failure to incorporate [3H]dThd cannot be considered evidence of non-S-stage position. Furthermore, these differences in cell dynamics and the varying responses to toxic agents reported by different investigators highlight the danger of extrapolating results obtained for a particular cell line to a universal conclusion for the behavior of mammalian cells.

For both exponentially growing and stationary-phase cells, there was no significant change in the shape of the dose-dependent survival curve regardless of whether drugs were dissolved in medium (which contains 20% fetal calf serum) or in HBSS. Also, similar responses were observed with respect to recovery from potentially lethal and sublethal damage independently of whether the nitrosourea agent was delivered in medium or in HBSS. These results disprove the contention (20) that serum-containing medium may change the true shape of the curve describing the lethal effects of nitrosoureas, at least for these human cells. Although the nitrosourea agent and some of its decomposition products may bind to serum proteins, it is apparent that this phenomenon does not affect its overall lethal efficacy or that not all of its degradation compounds are thus bound. It is possible that decomposition of nitrosoureas originates a sequential spectrum of compounds, all of which may possess some killing potential. For many cell types, the lethal mediators may be the initial products of the series only, and these may possess a high affinity for serum proteins. The lethal activity of the terminal products (which may not bind to serum proteins) is manifested only on cells sensitive to these specific products. Such a scheme would explain differences in lethality as a function of solvent noted for rodent cells with respect to human cells. Yet, Wheeler et al. (40) insist that BCNU, and not a series of its breakdown products, is the lethal agent, based on the fact that in their experiments incubation of BCNU for 30 to 60 min before cell treatment did not yield increased lethality over that obtained with incubation periods of only 30 min. Since nitrosourea breakdown yields products with very short half-lives (29, 38), it is possible that after 30 min incubation the sequential conversion proceeds to the point where active compounds transform into products that are inactive for the rat brain tumor cells used by these investigators.

LoVo cells failed to recover from potentially lethal damage induced by both nitrosoureas, but in split-dose experiments, these cells, in both exponential and stationary phase of growth, had a remarkable capacity to repair sublethal damage induced by both nitrosoureas. For BCNU, repair of sublethal damage is a fundamental difference with respect to results obtained for human lymphoma cells (15) and for CHO cells (5) where split-dose treatment actually increased the killing effect of nitrosourea drugs. However, the human lymphoma cells had shown complete recovery from the sublethal damage induced by cis-acid (14), and we had postulated that degradation of this agent did not yield 2-chloroethyl isocyanate, a decomposition product known to inhibit repair of DNA single-strand breaks (25). In the light of our present results, it appears that the answer is not that simple. BCNU does originate 2-chloroethyl isocyanate, and yet it failed to inhibit the recovery of LoVo cells. Thus, either this degradation compound is not responsible for inhibition of recovery or different cell types accomplish recovery
by different mechanisms, and only some of these are sensitive to the decomposition products of the nitrosoureas. Be it as it may, our results suggest that, in the treatment of large bowel cancers, nitrosourea derivatives should be less efficacious in reducing tumor burden if administered in fractionated schedules than when given as a bolus.

We also investigated the lethal effects of both BCNU and cis-acid as a function of cell position in the cycle at the time of drug administration. Both agents killed cells in all stages of the cycle with no major stage-dependent increase in efficacy. Examination of Chart 6 readily reveals that the response of cells is a function of tissue type rather than specific nitrosourea derivative. Thus, a similar survival pattern was obtained for both BCNU and cis-acid when the target cells were colon carcinoma, a pattern that differed significantly from that obtained for lymphoma cells. However, the responses of lymphoma cells to both agents were largely similar. Again, these results demonstrate the inadequacy of extrapolating results obtained on a given cell line to a generalized statement for the response of mammalian cells.

Another interesting difference of LoVo cells, with respect to lymphoma cells, was observed when they were incubated with the nitrosoureas for extended periods of time. For lymphoma cells, cis-acid had been so effective that even at a concentration of only 5 μg/ml the drug was capable of sterilizing 10⁷ cells, the lower limit of our assay system, in less than 4 hr incubation. Furthermore, its lethal effect in aqueous solution was stable for long periods of time. On LoVo cells, killing by cis-acid was less effective as a function of time while the lethal effect of the drug in solution decreased by 50% after 12 hr. In the case of BCNU, differences between cell lines were just as dramatic. Whereas on lymphoma cells BCNU had been completely ineffective after 4 hr in solution, on LoVo cells it exerted a progressively greater lethal effect for prolonged intervals, and the drug was almost as effective in solution after 24 hr as freshly prepared drug. These results suggest that nitrosourea derivatives might be considered candidates for treatment strategies for colorectal cancer that use continuous i.v. infusion. Perhaps this cannot be accomplished with BCNU because of its solvent requirements, but the ready formulation of cis-acid in aqueous solution may render this compound the agent of choice if such modality is ever implemented.

The lethal activity of nitrosourea derivatives is believed to be mediated by highly reactive breakdown products with relatively short half-lives (29, 38) yielding terminal compounds with no lethal efficacy. However, this drug decomposition pathway has usually been investigated in cell-free systems. Our studies afford the possibility of evaluating the contribution of cellular activity on the decomposition mechanisms of these agents. One possibility is that cellular products of line LoVo (perhaps enzymes?) block, transform, or otherwise modify carboxamylating and/or alkylating decomposition compounds of nitrosourea, thus originating the differential effects noted for the different target cells as the incubation interval is prolonged. It is also possible that the spectrum of nitrosourea degradation products is greater than that presently suspected. For many cell types, only the initial products may be lethal, while the terminal compounds are relatively innocuous. For other cells, all or only the terminal compounds exert lethal activity. Hence, for such cells, although the nitrosourea can no longer be identified chemically, its biological effect (i.e., cell kill) can be demonstrated readily by survival studies. Experiments currently under way intend to further investigate this phenomenon by cross-reacting BCNU-containing supernatants from LoVo and lymphoma cells followed by colony formation.

Based on the results of our investigation using an established line of human colon carcinoma cells, it could be concluded that nitrosourea derivatives should be excellent agents for the systemic treatment of colorectal cancer with an efficacy at least similar to that obtained in the therapy for human lymphoma. Yet, these conclusions have not been confirmed by Phase 1 and 2 clinical trials with these drugs (11, 28, 37). From the studies of Leibovitz et al. (28), we know that cultured colon carcinoma cells display differential morphological, cytogenetic, and carcinoembryonic antigen production properties that permit classification of such cells into 3 different groups. Preliminary studies currently performed in our laboratory, using representatives of these 3 groups, indicate that these differences extend to other properties including growth kinetics parameters. Possibly, these differences may be reflected by differential responses to a given antitumor drug. This possibility is supported by the demonstration of differential sensitivities to several antitumor agent of various permanent cell lines, all derived from rat hepatoma (2); by the distinctiveness in response of different human colorectal tumor xenografts to a spectrum of chemotherapeutic agents (24) and, more dramatically so, by the differential survival elicited on 4 sublines, all derived from the same human melanoma nodule by 1-β-D-arabinofuranosylcytosine (3) and by bleomycin (1). LoVo cells, which represent Group 1 of Leibovitz’ classification, may display the response of a unique subgroup of colon carcinomas which may encompass the spectrum of responses observed in clinical trials. Thus, it is possible that a reevaluation of the role of nitrosourea derivatives in colorectal cancer, based on the specific biologic properties of the cancer cells, may permit the selection and isolation of a unique group of patients that can be benefited with this form of therapy.

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REFERENCES

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