Letter to the Editor

Cell Survival following Treatment with Antitumor Drugs


Tsukeda et al. (13) recently published a report detailing experiments where the presumed cytotoxic action of several antitumor agents was measured by a dye exclusion test. Cells were continuously treated with the agents for periods of 4 to 6 days and stained with 0.2% nigrosin every 12 hr. Cell viability was evaluated as a function of dye incorporation. These investigators claimed that their method was adequate and appropriate because: (a) it was simple and rapid; (b) it did not require additional treatment that may affect the results; and (c) cells were continuously exposed to drugs for 4 days allowing estimation of cumulative cell death.’’ They further maintained that, although the colony formation technique used by many investigators to assess drug-induced cell lethality might be more reliable than the dye exclusion technique, it had the disadvantage of varying plating efficiencies among different cell lines.

It is unclear how these arguments can validate the obsolete dye exclusion method for cytotoxic assays. While the disadvantage of varying plating efficiencies among different cell lines can be internally corrected in reference to untreated control cells, the speed and simplicity of the dye exclusion method cannot amend the inaccuracies of results. It is also incorrect that cells used for dye exclusion do not undergo traumatic manipulation as much as such cells must be harvested, either by scraping or by enzymatic treatment, before counting in a hemocytometer. Furthermore, continuous incubation with drugs is an artificial situation far removed from most clinical situations where the majority of the agents are given as a bolus treatment. This results in an effective plasma level of only 30 min to, at most, a few hr. Any lethal effect induced by these drugs has to be exerted during this short period of time. Thus, continuous incubation in vitro, while capable of demonstrating ineffective drugs, may lead to false results for agents which, although potentially able to reduce tumor load in i.v. infusions, are ineffective in a ‘push’ modality. For drugs with rapid degradation (i.e., nitrosoureas) where the biological effectiveness may be rapidly lost (5), continuous incubation appears superfluous.

Cancer is primarily a disorder of cell proliferation (4). Because antitumor therapy is oriented towards suppressing this untoward cell proliferation, relevancy of results concerning cytotoxicity of an antitumor agent can be defined only in terms of decreased cellular reproductive capacity. Drug-induced cell killing is the result of an interplay between the type, extent, and duration of the damaging effect caused by a drug to critical biosynthetic pathways or subcellular structures and the capacity of living elements to bypass or repair such damage. Thus, several investigators have documented that a lethally damaged cell may not only complete DNA synthesis, but may even divide several times before the entire progeny perishes from the lethal damage inherited from their single ancestor (6, 7, 10, 12, 15). Conversely, cells showing severe metabolic and kinetic alterations immediately following drug exposure may recover and subsequently proliferate as if they had never been exposed to an injurious agent (1, 17). Hence, for proliferating populations, the lethal effects of an agent must be defined by its impairment of the reproductive integrity of the individual cells. In vitro, this impairment can be assessed only by the inability of cells to proliferate indefinitely, forming colonies under the appropriate experimental conditions (8).

Waters and Hofer (14) and Yuhas et al. (18) have independently documented the inability of dye exclusion tests to assess accurately the reproductive capacity of cells separated by physical means or by strong enzymatic treatment. About 2 years ago, we published a report demonstrating that tests measuring cellular metabolic derangement (i.e., dye exclusion, labeling index, rate of [3H]thymidine incorporation, and 51Cr release) following brief treatment with antitumor drugs (1 hr) failed to correlate with results obtained by the colony formation technique (9). Our results were confirmed by independent studies simultaneously published by Bhuyan et al. (2). These observations prompted an investigation designed to evaluate possible correlation between these techniques as the drug incubation interval was prolonged. The data from these experiments were never published; however, the results may settle the controversy as to which methodology is the most appropriate to evaluate drug-induced cell lethality.

As target cells, we used a human lymphoma cell line (T cells), the biological and growth kinetics characteristics of which have been extensively investigated in our laboratory (3). T cells were exposed to 3 concentrations of both adriamycin (0.1, 0.5, and 1.0 μg/ml) and bleomycin (25, 50, and 100 μg/ml) for periods up to 8 and 30 hr, respectively. 1,3-Bis(2-chloroethyl)-1-nitrosourea was not investigated because its biological activity degrades rapidly (5). Doubling times, incorporation of [3H]thymidine (labeling and scintillation indices), and dye exclusion tests were conducted as previously described (9). Effects observed for bleomycin-treated cells demonstrated a lack of correlation between results obtained by the above-mentioned methods and those of colony formation (Chart 1). After 30 hr, while cell kill estimated by colony formation was greater than 3 decades, the values determined by [3H]thymidine incorporation, under the assumption that a cell that incorporates DNA precursors is viable, were only 1 decade, and cell kill

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measured by dye exclusion was negligible. Similar results were noted for cells treated with 25 and 100 μg/ml.

Chart 1. Comparison of results obtained following continuous treatment of T, cells with bleomycin, (50 μg/ml). The surviving fraction defined by the colony formation technique (left ordinate) is contrasted with percentage of reduction of control values (untreated cells) for dye exclusion (trypan blue and eosin) and tests that measure incorporation of [3H]thymidine [scintillation index (S.I.) and labeling index (L.I.)] on the right ordinate. Similar results were noted for cells treated with 25 and 100 μg/ml.

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12. Thompson, L. H., and Suit, H. D. Proliferation kinetics of x-irradiated tumor cells with bleomycin, (50 pg/ml). The surviving fraction defined by the colony formation technique (left ordinate) is contrasted with percentage of reduction of control values (untreated cells) for dye exclusion (trypan blue and eosin) and tests that measure incorporation of [3H]thymidine [scintillation index (S.I.) and labeling index (L.I.)] on the right ordinate. Similar results were noted for cells treated with 25 and 100 μg/ml.

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Experimental results indicate that normal and cancer cells respond differently to changes of several environmental conditions suboptimal for growth. Normal cells rapidly enter a resting state following growth and remain viable for an extended period of time. On the other hand, under similar restrictive conditions, transformed and cancer cells continue to grow and finally die. Thus, it is proposed that normal cells have the control mechanism through which proliferation is regulated, and cancer cells have lost this control mechanism. It may be possible, therefore, that normal cells in which reproductive integrity is impaired following drug exposure remain viable for a relatively long period of time, but cancer cells do not remain viable and die off more rapidly.

The colony formation method does not assess such viable cells with impaired reproductive capacity. Although the dye exclusion test is less quantitative and underestimates cell lethality compared with the colony formation method, it may be useful for assessment of such metabolically viable but reproductively impaired cells, which may be especially important for evaluation of the differential cytotoxic action of antitumor drugs on normal and cancer cells. This differential effect of drugs could be more remarkable in several antimetabolites since those agents create nutritional defi-

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