Antineoplastic Drug Screening

I wish to respond to the recent editorial in the Journal by Dr. Larry Weisenthal (1) concerning laboratory screens for antineoplastic drugs. His point that more time and resources should be devoted to the evaluation of different types of screening tests is well taken. I also agree that the choice of assay end point is critical. While the biological end point (i.e., reproductive capacity versus growth inhibition) may differ, however, the actual assay end point is the same for the tests he describes; that is, agents are compared by inhibitory concentration, commonly the IC50 (i.e., drug concentration reducing growth to 50% of control). This end point ignores the pharmacodynamics of drug action, where cellular effects are a complex function of both concentration and time. Our experience developing the large arylmethylamino propanediol (AMAP) series of antitumor agents is a case in point. We evaluated more than 400 analogues in the standard P388 leukemia cell in vivo screen and were unable to correlate activity in vivo with any in vitro assay based on IC50, whether the biological end point was chromium release, thymidine labeling, colony formation, or mitochondrial metabolism. Subsequently, I discovered some early work with bacterial disinfectants, where drug action was found to be a function of exposure defined as C^n x T = k [reviewed in (2)]. The concentration coefficient n can range from less than one to as much as six, which argues that agents should be compared by an exposure parameter, rather than by concentration. Therefore, I developed the pharmacodynamic assay, which has an exposure end point, termed the minimum C x T, that accounts for variation in n (3). Evaluation of representative AMAPs in P388 leukemia cells in vitro by pharmacodynamic assay gave a good correlation to in vivo activity, provided a correction was made for host toxicity (4). This work also demonstrated that (a) all AMAPs and most of the established agents tested obey the C^n x T = k principle, (b) growth inhibition, not cytotoxicity, is the appropriate biological end point, (c) analogues with only subtle structural differences (isomers) can be discriminated, and (d) activity profiles in P388 leukemia are distinctly different from those in models for solid tumors and normal cells.

As Dr. Weisenthal (1) rightly argues, any in vitro test should be validated on the basis of how well the assay reflects the clinical activity spectra of established antineoplastic drugs. It is surprising that most of the current in vitro assays, including the National Cancer Institute screen, have not addressed this point. One reason may be that, with solid tumors in particular, there are no single agents with adequate activity to use as "gold standards." The best case may be breast cancer, where new drugs such as vinorelbine (Navelbine) are showing promising activity (5). To test the validity of the pharmacodynamic approach, six drugs with a range of activity in breast cancer were assayed in MCF-7 cells, a human breast cancer line that exhibits a chemosensitivity pattern consistent with observed clinical response (6). The results shown in Fig. 1 demonstrate a reasonably good correlation between in vitro activity and clinical response, with a two-log difference in minimum C x T/LD50 (i.e., 50% lethal dose value) between the most active and inactive agents. While these results may not be definitive, they suggest that consideration of pharmacodynamics in assay end points may improve existing in vitro screens for new cancer drugs.

References


Fig. 1. Correlation of pharmacodynamic assay to clinical activity in breast cancer. Clinical agents cisplatin (cDDP), etoposide (VP-16), vincristine (VCR), fluorouracil (5-FUra), doxorubicin (DOX), and vinorelbine (Navelbine) (NVB) were evaluated by pharmacodynamic assay in human MCF-7 breast cancer cells by methods previously described (3). Results are expressed as the minimum C x T required to obtain a surviving fraction of 2 (versus 5-6 for untreated controls) divided by the murine LD50 (mmol/kg). Clinical response rates are those previously reported (5,6). Solid line: least squares linear regression fit of the data (R^2 = .91). Broken line: 95% confidence bands.

Response

Dr. Adams agrees with my contention (1) that much more effort should be devoted to evaluating, understanding, and improving different types of cell culture assays. My editorial was mainly concerned with fresh tumor assays. In particular, I found it lamentable that "we still don't know if fresh-tumor assays can tell us anything important that cell lines can't" and we still don't know which types of fresh-tumor assays can give us the best information.

Adams goes on to cite data and to make a number of statements based on studies carried out in two well-established cell lines (P388 cells and MCF-7 cells). He quotes and describes some of his innovative work to develop a novel end point for in vitro studies using established cell lines. This work seems quite worthy of further study to determine if the "minimum C x T" does indeed offer advantages over the IC_{50} for given applications.

I did not limit biologic end points to "reproductive capacity versus growth inhibition." Many other end points, e.g., structural and metabolic alterations, have been and are being applied to cell culture studies in fresh tumors in which cell growth and reproductive capacity are often not measurable (2). Also, comparison of agents is not limited to the inhibitory concentration required to produce a given effect (commonly IC_{50}) but also to the magnitude of effects produced at given concentrations (which is a related but not identical end point). These unconventional biologic and assay end points are quite capable of modeling and reflecting many aspects of clinical chemotherapy (2-6) to a degree which has not been reported for more traditional cell proliferation end points.

A number of Dr. Adams' statements are, moreover, a little misleading. His findings regarding the lack of correlation between inhibitory concentrations in vitro and P388 activity in vivo are contrary to those reported earlier by other investigators (7). His statement that "growth inhibition, not cytotoxicity, is the appropriate biological end point" refers to his studies in P388 cells showing that the "minimum C x T" end point correlated with short-term survival in mice with P388 leukemia only when in vitro tests were carried out at drug exposures where the surviving cell fraction, relative to time zero plating controls, was greater than 1 (meaning that there was a net increase in cell numbers in drug-exposed cultures during the course of the assay). These slight inhibitions of exponential growth in vitro correlated with short-term survival (1-2 week) survival but did not correlate with long-term survival in mice with P388 leukemia (8). In contrast, assays based on cytotoxicity end points have been found to correlate with both short-term and long-term survival in humans (6, 9, 14).

Finally, clinical oncologists will probably note that the correlations between in vitro activity in MCF-7 cells and clinical response rates shown in Fig. 1 in Dr. Adams' letter would not be nearly so clear-cut were the abscissa values revised to reflect more recent data from clinical trials. In particular, cisplatin and etoposide are probably much more active in the clinic than they are shown to be in Dr. Adams' Fig. 1 (15, 18).

The details of my disagreements with Dr. Adams are less important than the lessons they illustrate. We all have a dreadfully inadequate knowledge of many very basic principles of cell culture assays. Are fresh tumors better than cell lines for specific applications? What are the best biologic and assay end points for specific applications? If fresh tumor assays have certain advantages, can they be made practical for drug development and clinical practice? Until we can answer such simple questions with facts, rather than with opinions, National Cancer Institute (NCI) program solicitations have no business offering such outrageous guidelines as "cell culture assays with cytotoxic end points that do not offer a distinct advantage over models in current use are not encouraged" (NCI Anticancer Model Development PA-91-90). The last decade has produced such a large volume of such a priori censorship in study sections that American investigation into cell culture assays using fresh tumors has been effectively extinguished, at least in research supported by the public sector. I am becoming convinced that drug screening will never be moved from the clinic into the laboratory until our patients begin to demand it, which they one day surely will.

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References

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