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**TITLE:** **Display** and **Analysis of Patterns of Differential Activity of Drugs Against Human Tumor Cell Lines:** Development of Mean Graph and COMPARE Algorithm

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ABSTRACT: The objective of this study was to develop and investigate an approach to optimally detect, rank, display, and analyze patterns of differential growth inhibition among cultured cell lines. Such patterns of cellular responsiveness are produced by substances tested in vitro against disease-oriented panels of human tumor cell lines in a new anticancer screening model under development by the National Cancer Institute. In the first phase of the study, we developed a key methodological tool, the mean graph, which allowed the transformation of the numerical cell line response data into graphic patterns. These patterns were particularly expressive of differential cell growth inhibition and were conveniently amenable to further analyses by an algorithm we devised and implemented in the COMPARE computer program.

**TEXT:**

The National Cancer Institute (NCI) is implementing a new anticancer drug-screening program using a disease-oriented panel of cultured human tumor cell lines for the initial stages of screening. More detailed aspects of this program and its origins, goals, methods, techniques, and rationale were described previously [n1-n4]. Criteria to define activity in the new screen are being explored. One criterion under study, differential cell growth inhibition, was the principal focus of our study.

Differential growth inhibition as defined here means that a cell line or group of cell lines can be inhibited to a given extent at a lower concentration of test drug than is required to exert the same effect on other cell lines. Differential growth inhibition has not been systematically investigated as a criterion for new drug selection. Therefore, unique data treatment and display methods were necessary for examination of its potential for determining activity in the new NCI drug screen. The objective of this study was development of methods to optimally detect, rank, and display test results that signal differential growth inhibition. A key result of the first phase of the study, the mean graph, has catalyzed development of additional methods by transforming the numerical cell line response data into graphic patterns that express differential growth inhibition.

Methods

We performed dose-response testing with cells from each cell line in a pilot screening panel. This panel consisted of [SYMBOL OMITTED]50 cell lines, including colon, lung, ovarian, and renal carcinoma; melanoma; central nervous system tumors; human leukemia; and a miscellaneous group of cell lines including the MCF-7 breast cancer and P388 murine leukemia cell lines and multidrug-resistant variants of these two lines. Test samples were prepared in dimethyl sulfoxide at their maximum soluble concentrations, diluted 1:200 with medium, and tested in culture at five log[10]dilutions.

Drug screening was performed with automated assay methods described previously [n2,n3]. It is important to note that use of a different assay method or end point determination could yield substantively different screening data profiles and interpretations. Nevertheless, our general approach to data display and analysis may be applicable to a variety of assays and/or end points.

## Results and Discussion

The large amount of data generated for each compound ([SYMBOL OMITTED]50 dose-response curves) required an optimal format for the graphic presentation of differential cell growth inhibition. The mean graph format developed for this purpose not only permits the ready visualization of any differential growth inhibition expressed by a test compound but also creates a framework for logical analysis of the data. This framework provides the foundation for computer-assisted analysis of the data, a vital development, considering the unprecedented scale of the NCI in vitro drug-screening program. However, the most intriguing property of the mean graph format is that it yields identifiable and characteristic "fingerprint" patterns, which appear to possess a remarkable degree of structure-function information.

### Mean Graph

The concept for the mean graph emerged in part from attempts to detect differential growth inhibition from a standard bar graph presentation. Indications of differential growth inhibition in a standard bar graph format reside only in the "ragged edge," the region between the tips of the bars for the least responsive cell line and the most responsive cell line. Figure 1 illustrates a typical horizontal bar graph and the corresponding mean graph. In this horizontal bar graph, the lengths of the bars are directly proportional to the potency of the test compound against the tumor cell line, which is expressed as the logarithm of the concentration resulting in 50% growth inhibition (IC[50]). The region between the baseline and the right end of the bar representing the least potent response (fig. 1A) tends to defeat the perception of differential growth inhibition. As an alternative, we developed a graph centered at the arithmetic mean of the logarithm of the IC[50] values for all cell line responses measured for a compound. While choice of the mean as an anchor point is arbitrary, its use has proven to be advantageous for the development of other mean-graph-derived analyses such as the estimation of relative cell line sensitivities [n5].

The mean graph (fig. 1C) is constructed by projecting bars to the right or left of the mean, depending on whether cell sensitivity to a test drug is more or less than average. The length of a bar is proportional to the difference between the logarithm of the cell line IC[50] and the mean. Differential growth inhibition is depicted by the bar (delta), which projects to either side of the mean. A bar projecting 3 log units to the right of the mean, for example, would reflect a cellular response 1,000 times more sensitive than the average of all of the cellular responses to the compound represented on the graph.

### Ranking by Degree of Differential Growth Inhibition

One approach to surveillance of the drug-screening data base is to rank compounds by their degree of differential growth inhibition. A novel technique for ranking follows from the mean graph format.

The first step in this approach is to identify a single best delta for a compound among all the deltas generated for it. This best delta is identified as Delta. The simplest definition of Delta is the highest numerical value of delta. However, a potentially better definition of the best delta is that delta representing the largest number of standard deviations from the mean of all the deltas observed for a cell line. Thus, Delta is the statistically rarest delta. The advantage of this statistical approach is that it helps to reduce the selection bias toward the intrinsically more sensitive cell lines. To obtain Delta by this method, one calculates the number of standard deviations from the mean of deltas that each delta represents.

The cell line-specific mean of delta values and the corresponding standard deviations are computed from the delta values for each cell line for all of the compounds evaluated. For a given delta, the appropriate mean is subtracted from the delta, and this difference is divided by the corresponding standard deviation. The quotient is the number of standard deviations from the cell line-specific mean. The means and standard deviations for each cell line are listed in table 1.

Table 1. Cell line-specific means, standard deviations, and confidence limits of delta*

[SEE ORIGINAL SOURCE]

The second step in this method is to rank compounds in order of their differential growth inhibition using the Deltas selected in the first step. Searching and sorting by Deltas provides an efficient means to identify compounds exhibiting differential growth inhibition.

Pattern-Recognition Algorithm

Another application of the mean graph format relates to pattern recognition. This format creates a characteristic fingerprint pattern for each compound. The possibility that these patterns might contain other exploitable information was investigated. We devised a simple algorithm that was implemented in the COMPARE computer program to rank the similarity of the mean graph pattern of a specified "seed" compound to the patterns of all the other compounds in the NCI screening project data base. Any previously tested compound can be used as the seed to initiate the pattern-recognition program.

The COMPARE program evaluates the similarity of mean graph patterns by computing average differences between the deltas obtained for a specified seed compound and the corresponding deltas for each of the other compounds in the data base. For a seed compound screened against a panel of 50 cell lines, the 50 deltas obtained for those cell lines are subtracted from the corresponding deltas obtained in testing the same 50 cell lines (or a subset of these lines if all 50 were not tested) against each of the other compounds in the data base. In this way, the mean graph pattern for the seed compound is sequentially compared quantitatively with all other mean graph patterns available. For each compound, two parameters are calculated: Av, which is the average difference between deltas (computed as the mean of the absolute values of the differences), and Max, which is the maximum difference observed.

The Av and the Max values are used by the COMPARE program to create a list of compounds ordered according to the similarity of their mean graph patterns to the mean graph pattern of the seed compound. The compounds are first sorted by Av values; compounds with lower Av values are ranked higher. Then compounds with the same Av values are further sorted by Max values; compounds with lower Max values are ranked higher. The purpose of creating the ordered list was to first rank the compounds by their similarity in mean graph fingerprints and then to investigate whether this similarity correlates with any other significant property common to the ranked compounds and the seed compound.

Since the meaning of the similarity in mean graph patterns was the central question, it was necessary to obtain additional information about the test compounds as well as relevant information about the seed compound. We constructed a data base of 88 test compounds suitable for this purpose and analyzed this data base with the COMPARE program. Application of the algorithm to data sets restricted to well-known or prototypical seed compounds led to intriguing results. Compounds known to be DNA binders (table 2), biological alkylating agents, or antimetabolites were grouped to a significant extent with agents having similar activity or structure.

Table 2. COMPARE pattern-recognition program: similarity of DNA binders and topoisomerase II inhibitors to doxorubicin *

[SEE ORIGINAL SOURCE]

In table 2, the seed compound was doxorubicin. The three compounds with the closest Av values were mitoxantrone, amsacrine, and acodazole. These three drugs and the seed compound are thought to be DNA binders and topoisomerase II inhibitors. The matching sequence includes taxol, rhizoxin, and then three more DNA binders (oxantrazole, bisantrene, and amonafide).

The COMPARE analysis using the alkylating agent melphalan as the seed for comparison showed a significant clustering of the known alkylating agents. Pipobroman, uracil mustard, and chlorambucil were ranked in that sequence as the closest matches to melphalan in the test set. The antimetabolite cytarabine was also used as the seed compound in a COMPARE analysis. Its close biological and structural analogue fazarabine ranked second to thioguanine in the listing.

There is an important caveat that applies to our algorithm. The algorithm must give a "best match" whether or not a meaningful one exists in the data base, and thus, unrelated compounds are sometimes given a high ranking. Despite this caveat, it seems that the observed mean graph patterns express valuable information, sometimes reflecting similarities in biological properties and/or chemical structure and properties. Expression of such similarities in the mean graphs appears to be sufficiently robust that this relatively simple algorithm of the COMPARE program can successful-

ly detect and rank these similarities in an order that seems to have an exploitable degree of correlation with independently derived rankings of similarity based on biochemical and/or structural considerations.

Conclusions

We have developed a set of computerized procedures to facilitate the detection , ranking, display, and analysis of patterns of differential growth inhibition. These procedures are conceptually centered in the mean graph, which was designed to graphically represent screening results for individual compounds tested against large numbers of tumor cell lines. Experimental applications of the COMPARE program to a limited data base accrued from the pilot screen suggest the possibility of meaningful clustering of mean graph patterns that is related to biological properties and/or chemical structure and properties. The potential wealth of information to be generated in the course of the new NCI drug-screening experiment can be subjected to a wide variety of analytical procedures. Our work represents only the first of many potential avenues to data display and analysis that may be explored in the course of this project.

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**GRAPHIC:** Figure 1, Comparison of two display formats for perception of differential growth inhibition in disease-oriented human tumor cell line panel. In standard horizontal bar graph, display of differential drug effects is confined to "ragged edge". Ready perception of differential effects in section 1B is effectively defeated by presence of large base section, 1A. Mean graph format, section 1C, facilitates perception of differential responses of cell lines by eliminating base section and projecting bars in opposite directions depending on whether cell sensitivity to the drug is more or less than average. Resulting fingerprint patterns provide delta values used in COMPARE analyses, LEUK = leukemia; NSCLC = non-small cell lung cancer, SCLC = small cell lung cancer, CNS = central nervous system; and MISC = miscellaneous. Range = No. of log units between values for most sensitive and least sensitive cell lines.