will equal the negative of the association-rate constant k_1. The intercept of the said plot will enable one to compute the dissociation-rate constant k_2 from knowledge of $q_\alpha, q_\beta, q_\gamma$, and D_γ.

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Pharmacodynamics of Chemotherapeutic Effects: Dose–Time–Response Relationships for Phase-Nonspecific Agents

WILLIAM J. JUSKO

Abstract □ Pharmacodynamic relationships were developed to characterize the necrobiotic effects of phase-nonspecific chemotherapeutic agents which attach irreversibly to cell receptors. The site of drug action is considered to be a specific body compartment, and target cell inactivation by the agent results from a bimolecular drug–receptor interaction. Turnover of cells is assumed to occur by natural synthetic and degradative processes. Based on these premises, a log–linear relationship was evolved to relate the fraction of surviving cells to the drug level–time integral at the pharmacologic site. The integral was shown to be proportional to the dose and independent of the mode of administration when the entire drug level–time course is evaluated. Data from the literature for the effects of cyclophosphamide on three cell systems of mice demonstrate the usefulness and certain therapeutic implications of the equations.

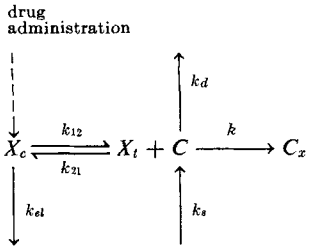
Keyphrases □ Pharmacodynamics—dose–time–response relationships, phase-nonspecific agents □ Chemotherapeutic agents, phase nonspecific—pharmacodynamic model □ Dose–effect relationships—cyclophosphamide on cell systems of mice

Considerable progress has been made in the development of kinetic relationships characterizing pharmacologic effects. Levy (1) showed that the intensity *versus* time course of many clinically observable pharmacologic effects may be described adequately by mathematical expressions based on the kinetics of drug elimination and on the established relationship between amount in the body and response. In turn, the simultaneous use of pharmacologic effect and pharmacokinetic data was shown to be an added dimension in the analysis of pharmacodynamic data (2).

The pharmacologic response to most drugs can be quantitated in a log dose–linear effect manner. Such a relationship is essentially derived from the postulation of reversible interaction between drug and receptor (3, 4). The reversibility aspect of this mechanism precludes application of most classical pharmacodynamic principles to therapy with certain antibiotics, antimetabolites, and alkylating agents. The cytotoxic effects of such agents are usually dependent on the irreversible or covalent incorporation of drug into cell metabolic sites or pathways (5). The lack of a mathematical basis for predicting the clinical effects of chemotherapy and the clinical difficulties in measuring such effects have been partly responsible for the uncertainty involved in the design of appropriate dosage modes and schedules for chemotherapeutic agents (4). The purpose of this report is to develop pharmacodynamic principles that may be of quantitative and predictive value in the therapeutic use of such drugs.

THEORETICAL

A basic pharmacodynamic model for the characterization of the effects of chemotherapeutic agents is shown in Scheme I. The drug is introduced into the central compartment (X_c) using a suitable mode of administration. The site of chemotherapeutic effect (X_t) is considered to be a homogeneous compartment separate from the central volume of distribution. First-order transfer-rate constants between the two compartments are k_{12} and k_{21}, and the elimination-rate constant is k_{el}. A portion of the dose of drug that reaches the pharmacologic site is involved in an irreversible reaction (rate con-



Scheme I

stant k) with receptors of the target cells, which ultimately produces mitotic arrest of these cells (C_x). It is assumed that: (*a*) viable cells increase in number at their natural mitotic rate (k_s), (*b*) viable cells are also subject to physiologic degradation (k_d), and (*c*) the number of cells is equal or directly proportional to the number of receptors.

Considering the bimolecular interaction of drug and cell receptor and the natural cell turnover, the rate of change in quantity of target cells (C) with time can be written as:

$$\frac{dC}{dt} = -k \cdot X_t \cdot C + k_s \cdot C - k_d \cdot C \qquad \text{(Eq. 1)}$$

where X_t is the amount of drug at the receptor site. If S_f is the fraction of surviving cells (C/C_0), then upon integration Eq. 1 becomes:

$$\log S_f = \frac{-k}{2.3} \cdot \int_0^t X_t \cdot dt + \frac{k_g \cdot t}{2.3} \qquad \text{(Eq. 2)}$$

where $k_g = k_s - k_d$, and which is the essential amount–time–response relationship. Since the amount of drug in the receptor compartment will vary with time due to administration and distribution factors, the integral portion of the equation is required (*Appendix*).

If a negligible fraction of the dose is consumed by the chemotherapeutic reaction or:

$$\int_0^t X_t \cdot dt \gg \int_0^t C_x \cdot dt \qquad \text{(Eq. 3)}$$

then the distribution of drug to the tissue site can be approximated by a two-compartment open model. For such a system, it can be shown (*Appendix*) that, independent of the mode of administration of drug,

$$\int_0^\infty X_t \cdot dt = \frac{k_{12} \cdot \text{dose}}{k_{21} \cdot k_{el}} \qquad \text{(Eq. 4)}$$

Therefore, if measurement of S_f is made after all potentially effective drug is eliminated ($t \rightarrow \infty$), substitution of Eq. 4 into Eq. 2 yields the useful relationship:

$$\log S_f = -K_s \cdot \text{dose} + k_g \cdot t/2.3 \qquad \text{(Eq. 5)}$$

where:

$$K_s = k \cdot k_{12}/2.3 \cdot k_{21} \cdot k_{el} \qquad \text{(Eq. 6)}$$

A lethality constant for each drug–cell system can be represented by:

$$\text{ED}_{90} = 1/K_s \qquad \text{(Eq. 7)}$$

where ED_{90} is the dose increment of drug required to reduce the fraction of surviving cells by one order of magnitude.

The exponential form of Eq. 5 is:

$$S_f = (e^{-2.3 \cdot K_s \cdot \text{dose} \cdot e^{k_g \cdot t}}) \qquad \text{(Eq. 8)}$$

If the drug is administered in a dosage regimen consisting of doses of D_0 at time intervals of τ, then the cumulative effect of n doses can be calculated from:

$$S_f^n = (e^{-2.3 \cdot K_s \cdot D_0 \cdot e^{k_g \cdot \tau}})^n \qquad \text{(Eq. 9)}$$

where cumulative time is equal to $n\tau$. Essentially complete cell loss will eventually occur if D_0 and τ are chosen so that:

$$(e^{-2.3 \cdot K_s \cdot D_0 \cdot e^{k_g \cdot \tau}}) < 1 \qquad \text{(Eq. 10)}$$

or $2.3 \cdot K_s \cdot D_0 > k_g \cdot \tau$, which fit the criterion of:

$$\lim_{n \rightarrow \infty} S_f^n = 0 \qquad \text{(Eq. 11)}$$

EXPERIMENTAL

The derived relationships can be illustrated using the data of van Putten and Lelieveld (6). These investigators obtained cell survival data in mice using colony-forming units of osteosarcoma cells, isogenic bone marrow stem cells, and chimaera spleen cells. A portion of the data obtained 18–24 hr. after intraperitoneal doses of cyclophosphamide was used in the present report.

RESULTS AND DISCUSSION

The essential dose–time–response relationship, which was evolved to quantitate the effects of chemotherapeutic agents, is Eq. 5. This equation predicts a log-linear relationship between the fraction of surviving cells and the dose of drug. The slope of the curve, K_s, will be directly dependent on the affinity of the receptor for the drug and the distribution and elimination properties of the active agent (Eq. 6). The curve will intercept the ordinate at an S_f value of unity if the generation rate of the cell system is small and the time interval between initial drug administration and cell survival measurement is soon after all active drug has been eliminated. Elimination may be due to the usual biotransformation and excretory processes; but for chemotherapeutic agents, it also may be the result of interaction of drug with cell constituents not required for the integrity or reproduction of the target cells.

The surviving fraction of cells, rather than the inactivated fraction, was chosen as the index of chemotherapeutic effect since it is usually possible to measure directly only the former (6). However, the lethality constant, ED_{90}, serves as a proportionality factor for comparing the cytotoxic effect of a drug on various cell systems or for comparison of effects of various drugs on a single cell system.

A linear log dose–effect relationship can be expected whether or not cell survival measurement is made before all effective drug is

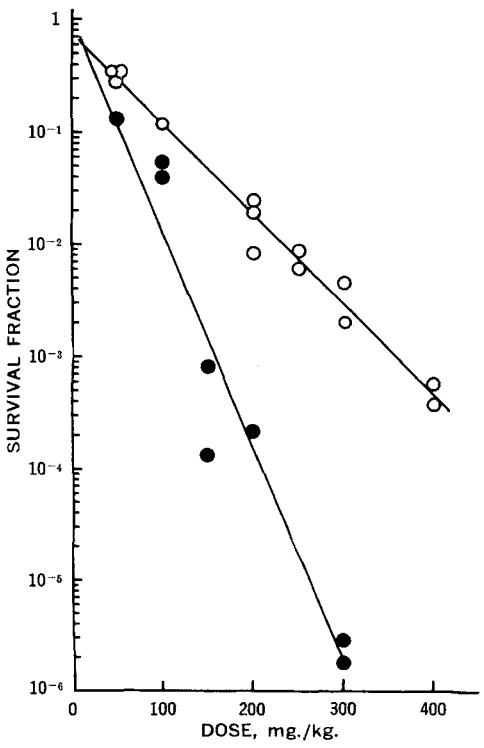


Figure 1—*Survival curves for chimaera spleen cells* (○) *and osteosarcoma cells* (●) *after intraperitoneal administration of single doses of cyclophosphamide to mice* (*data from* Reference 6).

Table I—Dose–Survival Parameters of Three Mouse Cell Systems after Cyclophosphamide Treatment

Cell Type	Mode of Drug Administration	Intercept, $k_g \cdot t/2.3$	Dose–Survival Constant, $K_s \times 10^3$	ED_{90} Dose, mg./kg.
Normal bone marrow	Single injection	0.4	3.43	292
	Multiple dose[a]	0.6[b]	2.88	347
Chimaera spleen	Single injection	0.8[b]	8.00	125
	Multiple dose[a]	0.5[b]	7.44	134
Osteosarcoma	Single injection	0.3[b]	1.88	532

[a] Four equal fractional doses at 2.5-hr. intervals. [b] Not significantly different from 1.0 ($p > 0.05$).

removed from the body. This is due to the proportionality of the drug level–time integral to dose at a specific time for each mode of drug administration. However, a constant and maximum slope, independent of the mode of drug administration, is obtained from dose–survival data only upon elimination of essentially all effective chemotherapeutic agent from the body. If cell survival analysis is performed prematurely, then the apparent slope value will underestimate the potential effectiveness of the drug.

Theoretically, it is possible to generate an apparent drug–receptor affinity constant, k, if actual tissue drug levels are directly measured. This experimental data could then be used to obtain the integral portion of Eq. 2 or to evolve the distribution- and elimination-rate constants in Eq. 6. Such pharmacodynamic refinements are most suitable for cell-culture systems, but they are also technically feasible for many tissue sites. The assumption that the receptors are contained within a homogeneous tissue compartment must be retained but on a more microscopic basis.

The log effect–dose relationship of Eq. 5 is essentially model independent. The necrobiotic effect of a drug will usually be proportional to the dose, regardless of the target site (*Appendix*). However, the proportionality constant, K_s, will vary with target site since the constant is dependent, in part, on the distribution of the agent to the site. Therefore, for drugs such as alkylating agents that bind strongly to cell receptors (5) and are usually eliminated rapidly, it is advantageous to utilize regional chemotherapeutic measures to increase distribution of the agent to localized or poorly perfused tumor sites (7, 8). This of course, also reduces exposure of the normal host cells to the dose of agent.

If the drug administered is a compound such as cyclophosphamide, which must be biotransformed to an active alkylating agent (9), then the dose factor of Eq. 5, assuming first-order kinetics, can be calculated from:

$$\text{dose} = k_f \cdot A^0/K_{el} \qquad \text{(Eq. 12)}$$

where k_f is the formation-rate constant for the alkylating agent, A^0 is the amount of precursor administered, and K_{el} is the rate constant for precursor elimination. A similar modification of Eq. 5 can be made for an absorption process where a constant fraction of nonparenterally administered drug might be considered as the dose.

Cyclophosphamide Data Analysis—Survival data for two cell systems of mice after treatment with intraperitoneal doses of cyclophosphamide are shown in Fig. 1. The sensitivity of the cell survival assay is demonstrated by the S_f values which extend over as much as six orders of magnitude. There is essentially a linear relationship between the logarithm of the fraction of surviving cells and the dosage of the drug as predicted by Eq. 5. The dose–response curves were similar in shape for vinblastine and radiation therapy (6). Furthermore, alkylating agents and radiation therapy produce log S_f–dose curves in *in vitro* cell culture systems which mimic the *in vivo* cell survival patterns (5).

Least-squares regression values for the data with cyclophosphamide were provided by van Putten and Lelieveld (6). These results were converted into the parameters of Eqs. 5 and 7 and are listed in Table I. The ordinate intercept values, except for one instance, did not differ significantly from unity. This indicates that the rate of generation of new cells (k_s) was not an appreciable factor in this set of data and that the time chosen to assay for cell survival (18–24 hr.) was sufficient to permit all drug to be eliminated. The latter is reasonable since, in mice, the alkylating metabolites of cyclophosphamide are known to be almost totally eliminated within 4 hr. of parenteral administration of 200 mg./kg. of drug (9).

The dose–survival constants (K_s) and lethality constants (ED_{90}) listed in Table I show marked differences for the three cell systems studied. These values, according to Eq. 6, reflect cell receptor differences in affinity for the drug as well as distribution factors responsible for drug accessibility to the pharmacologic sites. Also shown in Table I are K_s and ED_{90} values obtained in two of the cell systems when the drug was administered in four fractionated doses. The small differences in effect between the two modes of drug administration were not statistically significant (6).

Therapeutic Implications—The chemotherapeutic effects of multiple-dose administration of drug can be quantitated with Eq. 8. To elicit remission of the target cell mass, it is necessary to choose the dose and dosing interval so that the rate of cell kill is greater than the rate of cell regeneration, as shown by Eq. 10. This relationship also can be applied in the converse manner to protect certain host cells. To prevent inordinate loss of the most sensitive normal tissue, the dose and time interval must reflect: $2.3 \cdot K_s \cdot D_0 < k_g \cdot \tau$, where the constants apply to the host cells. The total number of doses required to eradicate completely the target cells will be dependent on the ability of a small number of such cells to resist other rejection mechanisms of the body.

The relationships evolved from the pharmacodynamic model (Eq. 5 and *Appendix*) predict that the *total* lethal effect of a given dose of drug will be independent of the mode of administration. This is, however, with the reasonable assumption that only a minute portion of the dose is sequestered at the receptor site. The relative usefulness of different modes of chemotherapeutic drug administration, such as slow infusion compared to rapid intravenous injection, has been a controversial question (10, 11). It is, therefore, of considerable interest that, as predicted by the model, subdivision of cyclophosphamide into four fractional doses did not significantly modify the dose–effect constants of the drug (Table I). Such results are most likely to occur for alkylating agents which are capable of inactivating cells during all phases of the mitotic cycle (5, 12).

These data and equations do not constitute a recommendation for an arbitrary mode of drug administration. A drug with side effects that can be quantitated in the classical log dose–effect manner should be given in fractionated doses. This is also true for chemotherapeutic agents that are cytotoxic during a limited phase of the cell mitotic cycle (12). And, not of least importance, biopharmaceutical factors may dictate use of a particular mode or route of administration.

The pharmacodynamic model proposed is a greatly simplified version of an extremely complex *in vivo* situation. Many factors can modify its direct application. For example, a general expression such as Eq. 5 will not be directly useful if the pharmacokinetics of the drug are dose dependent, if the affinity of the receptors for the drug change with time, or if distribution parameters of the target cells are perturbed during the course of drug therapy. However, the evolved mathematical relationships should prove of value for initial estimation of drug dosage schedules which maximize chemotherapeutic effect and reduce the incidence or severity of undesired effects. In addition, it is likely that a similar pharmacodynamic approach can be used to characterize the effects of other pharmacologic agents which elicit their effects through an apparently irreversible reaction with cell receptors.

APPENDIX

Definition of Tissue Level–Time Integrals—With the assumption that the receptors take up a negligible amount of drug, the time course of drug levels in compartment X_t after rapid intravenous injection can be written (10, 13) as:

$$X_t = \text{dose}\,(C_1 \cdot e^{-\alpha t} + C_2 \cdot e^{-\beta t}) \qquad \text{(Eq. A1)}$$

where $C_1 = k_{12}/(\beta - \alpha)$, $C_2 = k_{12}/(\alpha - \beta)$, and $\alpha \cdot \beta = k_{21} \cdot k_{el}$. The Laplace transform of Eq. A1 is:

$$L\{X_t\} = \text{dose}\left(\frac{C_1}{s + \alpha} + \frac{C_2}{s + \beta}\right) \qquad \text{(Eq. A2)}$$

or

$$L\{X_t\} = \text{dose} \cdot g(s) \qquad \text{(Eq. A3)}$$

If, instead of the unit dose impulse, drug is administered by a time-dependent transfer function, $F(t)$, which has a transform, $f(s)$, such that[1]:

$$\lim_{s \to 0} f(s) = \text{dose} \qquad \text{(Eq. A4)}$$

then Eq. A3, upon convolution of the transfer function, becomes:

$$L\{X_t\} = g(s) \cdot f(s) \qquad \text{(Eq. A5)}$$

Since it can be shown from Laplace theory that (13):

$$\int_0^\infty X_t \cdot dt = \lim_{s \to 0} \left[\left(\frac{C_1}{s + \alpha} + \frac{C_2}{s + \beta} \right) \cdot f(s) \right] \qquad \text{(Eq. A6)}$$

then evaluation of the limit, including substitution of Eq. A4 as well as for C_1, C_2, and $\alpha \cdot \beta$, yields the solution:

$$\int_0^\infty X_t \cdot dt = k_{12} \cdot \text{dose} / k_{21} \cdot k_{el} \qquad \text{(Eq. A7)}$$

which is also shown as Eq. 4 in the text.

Equation A7 was evolved previously by Gibaldi (10) for the instances when drug is administered by rapid intravenous injection and by constant-rate infusion. With a general proof similar to that presented here, however, it can be shown that for any multiple-compartment model describable by linear first-order differential equations with constant coefficients, each drug level–time integral (time $0 \rightarrow \infty$) will be independent of the mode of drug administration and proportional to the dose administered.

Time-Dependent Integrals—If it is necessary to use the time-dependent integral of Eq. 2, the specific equations must be evolved to characterize each mode of drug administration. Applying the Laplace theorum (13):

$$L\left\{ \int_0^t X(t) \cdot dt \right\} = X(s)/s \qquad \text{(Eq. A8)}$$

to the transform for the two-compartment system with rapid dose input (Eq. A2) yields:

$$L\left\{ \int_0^t X_t \cdot dt \right\} = \frac{\text{dose} \cdot C_1}{s(s + \alpha)} + \frac{\text{dose} \cdot C_2}{s(s + \beta)} \qquad \text{(Eq. A9)}$$

for which the antitransform is the integral:

$$\int_0^t X_t \cdot dt = \frac{\text{dose} \cdot C_1}{\alpha}(1 - e^{-\alpha t}) + \frac{\text{dose} \cdot C_2}{\beta} \times (1 - e^{-\beta t}) \qquad \text{(Eq. A10)}$$

This relationship can be substituted into Eq. 2 to calculate the time course of chemotherapeutic effect of a single intravenous dose of drug, providing the distribution and elimination constants of the system are available.

If the transfer function involves first-order input (*i.e.*, drug absorption) with the rate constant k_a:

$$F(t) = k_a \cdot \text{dose} \cdot \exp(-k_a \cdot t) \qquad \text{(Eq. A11)}$$

then the transform is:

$$f(s) = \frac{k_a \cdot \text{dose}}{s + k_a} \qquad \text{(Eq. A12)}$$

Convolution of Eqs. A12 and A3 and utilization of the principle of Eq. A8 yield the integral:

$$\int_0^t X_t \cdot dt = k_a \cdot \text{dose} \cdot C_1 \left[\frac{1}{\alpha k_a} + \frac{e^{-\alpha t}}{\alpha(\alpha - k_a)} - \frac{e^{-k_a \cdot t}}{k_a(\alpha - k_a)} \right] + k_a \cdot \text{dose} \cdot C_2 \left[\frac{1}{\beta k_a} + \frac{e^{-\beta t}}{\beta(\beta - k_a)} - \frac{e^{-k_a \cdot t}}{k_a(\beta - k_a)} \right] \qquad \text{(Eq. A13)}$$

Equations A13 and A10 may be useful for model-simulation purposes but, needless to say, are rather cumbersome to use in evaluation of experimental data. It is, therefore, advantageous to analyze chemotherapeutic effect data in terms of the total effect of a given dose of drug.

[1] For example, see Eq. A12.

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