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Laboratory Prediction of Clinical Chemotherapeutic Drug Resistance: a Working Model Exemplified by Acute Leukaemia

Rolf Larsson and Peter Nygren

Recent research into mechanisms of resistance to chemotherapy in acute leukaemia has been focused on various protective mechanisms at the cellular level, such as drug uptake, efflux, metabolism and DNA repair mechanisms, while therapeutic failures due to other potentially important causes have received relatively little attention. Here we describe a simple model to account for three major determinants of tumour response in acute leukaemia: cellular drug sensitivity, leukaemic cell regrowth potential and systemic drug exposure. Possible ways of measuring these parameters are discussed. It is suggested that laboratory estimation of these parameters may provide valuable information on clinical drug resistance and may help to design more adequate treatment strategies for the individual patient.
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INTRODUCTION

THE CLINICAL observation of apparent resistance to chemotherapeutic drugs is a frequent cause of treatment failure in acute leukaemia. At the cellular and molecular level, several potentially important mechanisms of resistance to anti-leukaemic drugs has recently been identified. Much interest has been focused on multidrug resistance (MDR) which denotes a broad resistance cellular phenotype to several structurally and functionally different drugs, including the vinca alkaloids, anthracyclines and etoposides. This type of resistance is believed to be mediated by the membrane-bound 170 kDa P-glycoprotein (Pgp) which actively extrudes the drugs from the cell [1, 2]. Atypical forms of MDR have been described and suggested to be mediated by alterations of a nuclear enzyme, topoisomerase 2 [1, 3]. Alterations in the cellular glutathione system, involving glutathione transferase-mediated conjugation of GSH with antineoplastic agents is another potentially important protective mechanism of the leukaemic cell [1, 4]. In addition, both non-Pgp-mediated cellular drug transport and subcellular distribution, as well as altered DNA excision/repair mechanisms, have been implicated in chemotherapeutic drug resistance [1].

Although important and suggestive, most of the above mentioned observations on mechanisms of cytotoxic drug resistance have originated from studies of laboratory-derived resistant cell lines. In the clinical setting there are clearly additional factors that may cause apparent resistance to chemotherapeutic drugs.

In the present commentary we propose a simple model to account for clinically observed resistance exemplified by acute leukaemia and suggest that laboratory estimates of these factors may help to design more adequate treatment strategies for the individual patient. The basic features of the model described are much influenced by the recent work of Preisler *et al.* [5, 6].

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THE MODEL

Chemotherapy of acute leukaemia is cyclic and sensitivity to a given treatment is usually determined clinically at "final" remission evaluation (> day 21 after beginning of therapy) after a defined number of courses (generally one to three) where complete and in some cases partial remission (CR and PR, respectively) indicate drug sensitivity. A patient with a residual leukaemic cell mass in the bone marrow at "final" remission evaluation in excess of the definitions for CR (or PR) is consequently judged as resistant or refractory, depending on previous response to chemotherapeutic drug regimens. For simplicity and clarity we restrict the graphical description of our model to only one course of chemotherapy and consider the attainment of CR as the strict measure of drug sensitivity. Also, for simplicity, the model assumes that the biological characteristics and drug sensitivity of the leukaemic cells remain constant over time. Treatment failure due to drug-induced non-tolerable toxicity is not considered in the model.

Figure 1 shows the principal features of the model. Curve a represents the cytolytic response of a CR patient during and

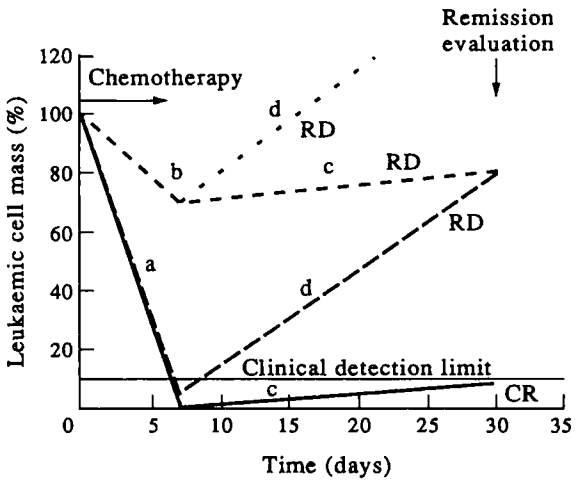

Fig. 1. Basic features of the model. Modelling the effect of induction therapy in acute leukaemia. See text for further explanation.

shortly after a course of chemotherapy. Curve b illustrates the comparably more shallow cytolytic response of a patient with resistant disease (RD). Curve c represents the regrowth phase of leukaemic cells typical of CR patients whereas curve d illustrates an increased regrowth rate typical of a RD patient. Although the shapes of these curves are illustrated conceptually, the principal features are based on published data from serial studies (day 0, 6, 17 > weekly) of the effect of chemotherapy on bone marrow content of leukaemic cells in patients with acute lymphocytic leukaemia, AML [5, 6].

According to this model only curves a–c will lead to CR at day 28 ("final" remission evaluation). Substituting the regrowth rate of the CR patient with that of the RD patient will offset the benefits of high initial cell kill and lead to RD outcome. Similarly, changing the initial cell kill rate of the CR with that of the RD patient will obviously lead to RD. Thus, the presence of any component of an RD curve, b or d, will result in RD outcome.

The principal factors that determine which of the curves will apply to the individual patient are depicted in Fig. 2. Whether the initial cell killing of leukaemic cells will follow a steep a curve or the more shallow b curve depends on intrinsic leukaemic cellular drug sensitivity (also called classical drug resistance) and/or the level of systemic exposure of the chemotherapeutic drugs, often characterised by the area under plasma concentration vs. time curve (AUC). The factors that determine which of the two curves c and d will apply is related to regrowth potential of the remaining leukaemic cells after chemotherapy which, in turn, may be determined by several biological and cell kinetic factors (see below).

We will now briefly discuss these three principle determinants separately and focus on possible ways of obtaining laboratory estimates in order to evaluate the clinical validity and applicability of the model.

CELLULAR DRUG SENSITIVITY

Large individual variations in tumour cell sensitivity to chemotherapeutic drugs among patients with the same tumour type remain a major obstacle to successful chemotherapy. The concept that most of this variation reflects underlying intrinsic sensitivity of the malignant cell is based on a number of studies correlating *in vitro* and clinical data [7]. Several non-clonogenic short-term (3–4 days) *in vitro* assays of fresh tumour cells have been employed for this purpose in many different tumour types

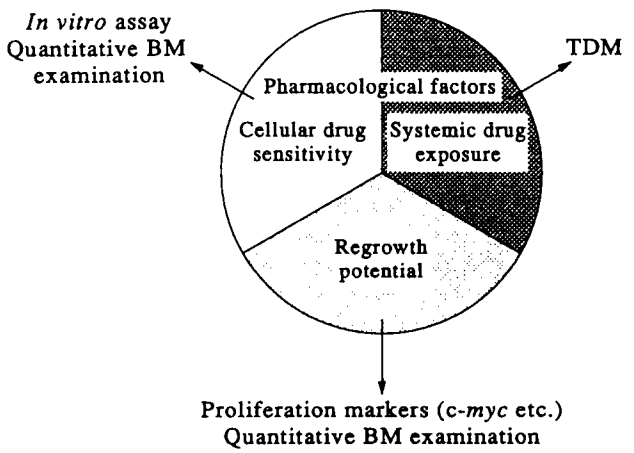

Fig. 2. Schematic illustration of the major determinants of clinical drug resistance. See text for explanation.

including acute leukaemia [8–13]. In the case of leukaemia, the most successfully used assays rely on the measurement of viability in the whole, largely non-dividing leukaemic cell population [7]. The morphologically based differential staining cytotoxicity (disc) assay has been the most widely used fresh tumour test in haematological malignancies [8, 13] but recently semi-automated microtitre plate-based assays measuring membrane integrity [10] or metabolic activity [9, 11, 12, 14] have also been evaluated. Overall, these *in vitro* assays have shown remarkably similar predictive capabilities, with sensitivities of 80–95% and specificities of 50–80%.

By using high drug exposure and use of improved calibration procedures it is now possible to further increase the specificity to > 99% with respect to the identification of extreme drug resistance in a subpopulation of patients [8, 15]. The theoretical, clinical and biological basis for these different *in vitro* tests, as well as their advantages and potential pitfalls, have been extensively reviewed by Weisenthal [7, 16] and is beyond the scope of this commentary. It should be noted, however, that the relatively high predictive accuracy for resistance is compatible with the current model suggesting intrinsic cellular drug resistance to be a sufficient hindrance for clinical response. The tendency towards a higher false positive rate (20–40%) compared with the false negative rate (5–15%) is also in accordance with the presence of clinical resistance due to other causes than cellular drug sensitivity. These non-clonogenic fresh tumour assays may thus be used for identifying patients with any type of intrinsic cellular drug resistance mechanism(s) in operation. The use of mechanistic markers (like Pgp expression, glutathione transferases, topoisomerase 2 expression, etc.) may be of less predictive utility since clinical drug resistance most likely is multifactorial [17] and many mechanisms are yet to be identified.

SYSTEMIC DRUG EXPOSURE

The dose intensity in chemotherapy may be of great importance for clinical outcome in many drug-sensitive tumours, including the leukaemias [18, 19]. This implies that reducing the dose to lower toxicity may seriously undermine the chance to achieve therapeutic responses in drug-sensitive patients. For the individual patient, however, interindividual variations in pharmacokinetic parameters like absorption, distribution and elimination of drugs also play an important role. The cytotoxic effect of most cytotoxic drugs is best related to the product of drug concentration (C) and exposure time (T) [18]. Clinically, C × T is represented by systemic exposure defined as the AUC. Most anti-leukaemic drugs display a large interindividual variation in systemic exposure (2–10-fold) also in patients with normal liver and renal function [18]. For certain drugs there is also an intra-individual variability between courses [20]. These facts make the systemic exposure for different patients receiving the same dose very unpredictable. This is illustrated in Fig. 3 where 2 hypothetical patients receive the same dose but in whom plasma clearance (CL) varies with a factor 2. This will lead to different concentrations (Cp) over T and consequently different AUC. These AUC will translate into diametrically different clinical outcomes when the concentration–response curve is steep (solid line). However, for a patient with leukaemic cells resistant to clinically achievable AUC (broken line) no difference in effect will be observed. An increase in AUC in this case will only add to toxicity.

The great heterogeneity among tumours with respect to cellular drug sensitivity makes measurements of AUC alone difficult to interpret and is probably a major reason for the lack

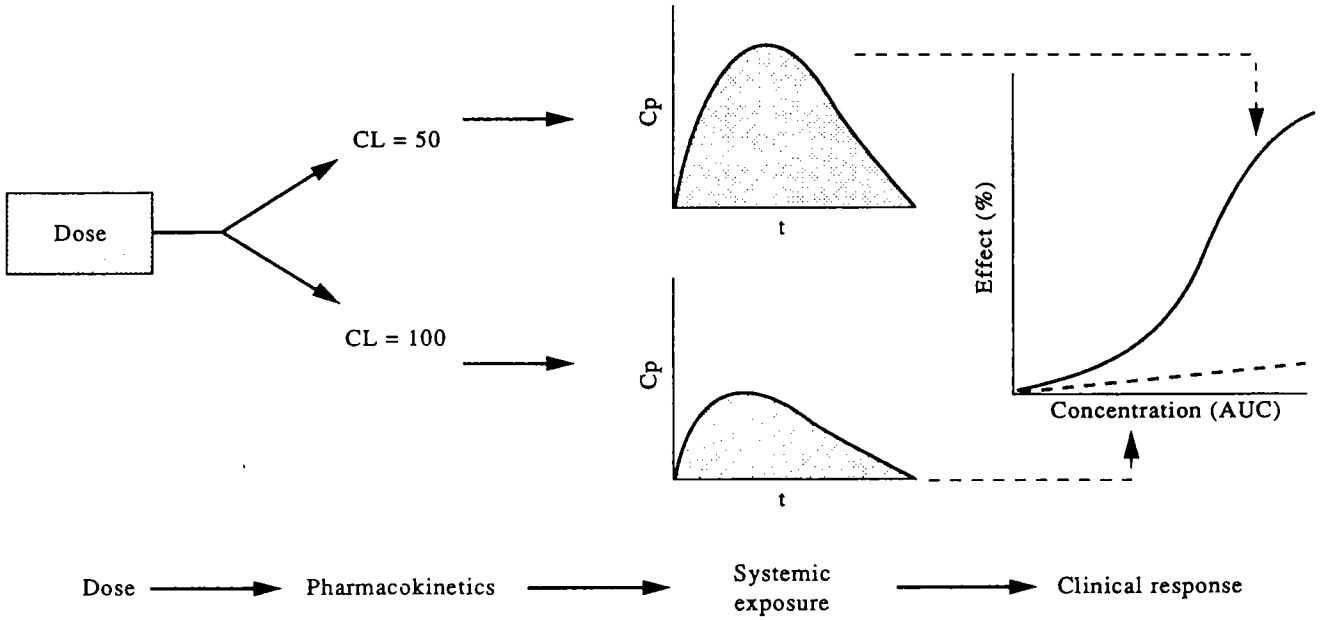

Fig. 3. Theoretical relationship between inter-individual pharmacokinetic disposition, systemic exposure and clinical response. Cl = Systemic clearance, Cp = plasma concentration, T = time, AUC = area under the plasma concentration vs. time curve (modified from [18]).

of success for therapeutic drug monitoring (TDM) in clinical oncology. This lack of enthusiasm for TDM is somewhat surprising considering the fact that for drug-sensitive patients with steep concentration–effect relationships, small changes in AUC may be of major importance for the final oncolytic activity achieved. Indeed, some studies in drug-sensitive tumours have demonstrated positive relationships between plasma concentration and therapeutic effect [21–23]. Nevertheless, the frequency of cases with suboptimal systemic exposures (pharmacokinetic resistance) for most tumour types is presently unknown. The idea that dose escalation to maximally tolerated dose (MTD) should prevent undertreatment is in our opinion somewhat utopic since the dose–toxicity relationship is seldom known and dose escalation based on laboratory or clinical parameters related to toxicity is, in fact, a rare clinical event. Even if dose adjustment to MTD is performed stepwise, there will be given an appreciable time between courses for drug-resistant subclones to emerge under suboptimal systemic exposure.

The situation becomes even more complex considering that combination therapy is often given and the component drugs often have similar dose-limiting toxicities, making judgement on which drug to escalate very difficult. Surprisingly, there is virtually no information on the variability and pharmacokinetic behaviour of drugs given in chemotherapeutic combinations. However, recent developments in analytical and statistical pharmacokinetics have made these areas of research more easy to address. Most important is the development of robust "limiting sampling" techniques allowing the investigator to estimate AUC from up to three timed blood samples, even in the case of bolus administration [24]. These practical improvements will hopefully lead to a more systematic investigation of the potential role of "pharmacokinetic" resistance in the clinical setting.

REGROWTH POTENTIAL

This factor is probably the most recently described clinical phenomenon that has been directly linked to drug resistance in AML [5, 6]. The extent of leukaemic cell regrowth between courses of therapy is probably determined by several factors: (a) the number of cells surviving chemotherapy, (b) percentage of cells in cycle, (c) proliferative potential (number of cell divisions each cell is capable of), (d) cell cycle time, (e) the ability to differentiate, and (f) presently undefined factors. The present model holds that even if the leukaemic cells are pharmacodynamically sensitive to the drug and high systemic exposure is achieved, treatment will fail if the surviving leukaemic cells regrow as fast as or faster than they can be killed.

Since this is a newly discovered clinical phenomenon, little information has yet been gained with respect to potential laboratory parameters which may be reflective of cellular regrowth. When describing leukaemic cell regrowth after therapy in AML, Preisler *et al.* [5, 6] used serial quantitative bone marrow examinations to measure both the level of cell kill and leukaemic cell regrowth after termination of therapy. The major drawbacks with this technique are the extra amount of work and patient inconvenience resulting from additional marrow samplings and the fact that results cannot be obtained prior to therapy. Using the expression of the proliferation-associated oncogene c-*myc* as a predictor for clinical outcome, Preisler *et al.* [25] suggested this marker to be reflective of proliferative potential in AML. High c-*myc* expression in the leukaemic cells was associated with short remission durations and CR could be obtained only in patients where a high degree of leukaemic cell kill had taken place [25]. Unfortunately, there appears to be some technical difficulties with immunohistochemical measurements of c-*myc* [26]. Measurements of different aspects of cell cycle kinetics or estimates of the proliferative characteristics of leukaemic progenitor cells using *in vitro* cloning may provide other possible alternatives for pretherapy predictions of regrowth potential [5]. *In vitro* cloning may, however, be less practical as a routine tool since many leukaemic cell samples will not establish clonal growth. Taking accurate estimates of regrowth potential into account may thus reduce the false positive rate of non-clonogenic *in vitro* tests of cellular drug sensitivity.

Table 1. Hypothetical description of predictions of tumour response based on laboratory estimation of the model parameters as well as clinical actions suggested to counteract the mechanisms of resistance

Determinant	Laboratory scenario A	B	C	D
Cellular drug sensitivity	High	Low	High	High
Regrowth potential	Low	Low	High	Low
Plasma concentration (AUC)	High	High	High	Low
Result	CR	RD	RD	RD
Action	Consolidation	Change drugs, add Pgp blockers	Shorten intervals, cytostatic or differentiating agents	Increase dose

MODEL INTEGRATION AND FUTURE DIRECTIONS

If it were possible to accurately predict cellular drug sensitivity, regrowth potential of leukaemic cells and, as early as possible after the start of chemotherapy, the expected systemic exposure for individual patients, patient-specific treatment regimens could be designed which hopefully would result in improved therapeutic outcome. This is conceptually illustrated in Table 1 where patient A shows a favourable profile with respect to cellular drug sensitivity, regrowth and systemic exposure and will be expected to enter CR. Patient B on the other hand shows evidence of classical drug resistance and should require a change of drugs or addition of resistance modifiers to increase the probability of response. Patient C shows a high cellular sensitivity to the chosen drugs, achieves high systemic exposures of the drugs but will fail therapy due to rapid leukaemic cell regrowth. In this case a change of schedule to minimise the time between courses or experimental manoeuvres designed to limit cell growth between courses (e.g. differentiation-inducing agents) may be appropriate. Patient D, finally, will achieve suboptimal systemic drug exposure which may be corrected by increasing dose.

What are then the current possibilities to measure the quantitative relationship of these parameters and evaluate the predictive ability of the model? As discussed previously, cellular drug resistance can be measured prior to therapy *in vitro* with nonclonogenic fresh tumour assays with relatively high accuracy [8, 15]. Alternatively, these measurements can be performed *in vivo* by serial bone marrow examinations [6]. Measurement of regrowth potential may require direct quantification of leukaemic cells in bone marrow samples [6] while other potential pretherapeutic measures (like c-*myc* expression and/or other proliferative characteristics of the leukaemic cells) are evaluated. Measurement of systemic exposure for all important anti-leukaemic drugs can currently be performed with existing analytical and pharmacokinetic technologies [18, 21].

APPLICATION OF THE MODEL FOR SOLID TUMOURS

In the present paper the proposed model has been described for treatment of acute leukaemia. Theoretically, the general principles may be equally applicable to solid tumours. Intrinsic cellular drug resistance may be measured equally well by nonclonogenic *in vitro* assays. Determination of systemic exposure may be performed in the same manner as in the leukaemic patients. However, for solid tumours the relationship between intrinsic cellular drug sensitivity and systemic exposure may be undermined due to contributing intratumoral factors (e.g. intratumour drug distribution due to tumour geometry, intratumoral pH, hypoxia, osmolarity, etc.) which may influence drug concentration and efficacy at the level of the tumour cell [27]. In these cases measurements of drug concentrations in the tumour area (or the tumour cells) may be necessary to obtain a complete account for the factors leading to clinical drug resistance. Determination of tumour cell regrowth between courses may be estimated by serial measurement using sensitive techniques with good time–effect resolution capabilities like positron emission tomography (PET) [28].

CONCLUDING REMARKS

In the present article we describe a simple model to account for, in our view, the major determinants of tumour response in leukaemia. It is also our belief that only taken together, these estimates may provide meaningful information. The experimental testing of the present working model will require collaboration between specialists from different fields, including clinical oncology, clinical haematology, pathology and clinical pharmacology. It is our hope that this simple working model (being proved true or false) may provide one example of a preliminary framework for more concentrated interdisciplinary efforts towards the understanding of the determinants of *clinical* (rather than laboratory) drug resistance in acute leukaemia and other forms of human cancer.

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Book Review

Cancer in Italy. Incidence Data from Cancer Registries, 1983–1987

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Cancer in Italy: Incidence Data from Cancer Registries, 1983–1987 represents a compromise between the two most popular ways of disseminating incidence data, i.e. "Cancer Incidence in Five Continents" edited every 5 years by the International Agency for Research on Cancer (IARC) and the local publication of individual Registries. Many reasons may have prompted the organisers of nine all-cancer-site registration schemes (i.e. six in North Italy, two in Central Italy and one in South Italy) in addition to two partial schemes (i.e. colorectal cancer and childhood neoplasms) to conceive such a publication.

The most notable is probably the need to summarise the impressive, although somewhat delayed (in respect to northern Europe) development of epidemiological research in Italy in the past 15 years or so. The number and the quality of Italian cancer registries emerge very well from the bulk of data presented and the richness of the comparisons and considerations on cancer trends and aetiology included in *Cancer in Italy*. In fact, in addition to many orthodox tables and figures, every cancer site is dealt with in detail in order to help the reader to link the picture which emerges from descriptive epidemiology to the present knowledge and hypotheses on cancer causation.

The second reason for this publication is related to the ways in which cancer registries have developed in Italy. In the absence of central planning, registries were born from the local initiative of a few epidemiologists, clinicians and pathologists. Therefore, as Max Parkin from IARC states in his opening remarks, "The task of producing a national synthesis is in Italy much greater than in compendia of results from registry networks in which pre-existing standardisation results from the explicit goal of unified analysis and publication (e.g. SEER-surveillance, epidemiology and end results, in the U.S.A.)". But also, I would add, more urgent. For good reason, aside from a very Italian emphasis on historical and political aspects of the growth of epidemiological research in Italy, the authors repeatedly state that this volume is part of a more general effort to improve data collection and epidemiological training.

I think also that *Cancer in Italy* constitutes useful reading for epidemiologists elsewhere. Indeed, Italy is an interesting "natural experiment" and still shows, in the period examined (i.e. mid-1980s), very substantial variations in the incidence of most individual cancer sites. The 2.4 ratio between the highest-incidence and the lowest-incidence area for cancer of the stomach and the almost 4-fold ratio for cancer of the oral cavity are examples of important opportunities of aetiological research.

Last, but not least, *Cancer in Italy* is entirely bilingual (Italian and English) and can be obtained for free by writing to Dr Zanetti (Registro dei Tumori per il Piemonte e la Valle D'Aosta, Unità di Epidemiologia, Dipartimento di Oncologia, Ospedale S. Giovanni Antica Sede, Via S. Francesco da Paola 31, 10123 Torino, Italy) or Dr Crosignani (Registro Tumori Lombardia—Provincia di Varese, Istituto Nazionale per lo Studio e la Cura dei Tumori, Via Venezian 1, 20133 Milano, Italy).

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