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Correlation between sensitivity *in vitro* of patient chronic lymphocytic leukemia cells and clinical systemic exposure at the maximum tolerated dose for cell cycle phase-specific (type 2) anticancer drugs

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Previously in *Anti-cancer Drugs* we have reported a high correlation between clinical plasma concentration–time products ($C \times T$) and the concentration of cytotoxic drugs giving 50% cell survival (IC_{50}) in primary cultures of human lymphatic cells. In the present study we investigated the relationship separately for cell cycle-specific (type 1) and cell cycle non-specific (type 2) drugs in chronic lymphatic leukemia cells. A high correlation ($R = 0.92$) was observed between $C \times T$ and IC_{50} for cell cycle non-specific drugs, while for cell cycle-specific, or $C \times T$-dependent, drugs, the relationship was much weaker ($R = 0.58$). Since the opposite pattern has been observed for the relationship between clinical $C \times T$ and LD_{10} in mice, these results further imply that drug sensitivity assays may be a useful complement to animal data in the selection of starting dose and dose escalation procedure in phase I clinical trials of new cytotoxic drugs.

Key words: Anticancer drug, exposure, maximum tolerated dose, sensitivity, type 2.

Introduction

The main purpose of the initial phase I trial of anticancer drugs is to evaluate the safety and determine the maximum tolerated dose (MTD) in man. The conventional phase I study usually starts at 1/10 of LD_{10} obtained in mice and the dose is then escalated to MTD by a modified Fibonacci scheme in cohorts of three patients.[1–3] Often numerous dose escalation steps will be required before the MTD is reached, and the phase I studies are consequently very costly and time consuming. Furthermore, there is an ethical problem since most patients enrolled are treated at ineffective doses. Indeed, the response rate in phase I trials is less than 5%.[4]

An alternative approach was suggested by Collins *et al.* in 1986. They retrospectively demonstrated a close relationship between the human concentration–time products ($C \times T$) at MTD and the mouse $C \times T$ at LD_{10} for several cytotoxic drugs, and proposed a pharmacologically guided dose escalation (PGDE) protocol, where the dose escalation is adjusted depending on how close the human $C \times T$ at the starting dose is to the target $C \times T$, i.e. mouse $C \times T$ at LD_{10}.[5] However, although proven feasible for several drugs, PGDE may not be suitable for drugs for which pharmacodynamic species differences are prominent.[6]

Recently, Fuse *et al.* reported that the reliability of PGDE may also be dependent on the cell cycle phase-specificity of a drug. A high correlation was demonstrated between mouse $C \times T$ at LD_{10} and $C \times T$ in humans at MTD for cell cycle phase-non-specific (type 1) drugs, for which the activity is dependent on the total drug exposure, $C \times T$. For cell cycle phase-specific (type 2) drugs, thought to depend on a certain time above a threshold concentration to be effective, there was a considerably weaker correlation.[7]

In *Anti-Cancer Drugs* we have previously presented an investigation of the relationship between cytotoxicity *in vitro* in drug sensitive tumors and pharmacokinetic estimates of systemic drug exposure at MTD in humans.[8] We found good correlations between the 50% cell kill concentration (IC_{50}) of cytotoxic drugs in primary cultures of human lymphatic cells and clinical $C \times T$ at MTD, suggesting that not only animal toxicology data but also data from studies on cytotoxicity *in vitro* may provide information valuable in the development of new cytotoxic agents. On the basis of the report on the high correlation between mouse and human $C \times T$ data for type 1 drugs,[7] we have now

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evaluated the relationship separately for type 1 and type 2 drugs.

Materials and methods

The IC_{50}s of 27 anticancer drugs were determined in primary cultures of fresh human chronic lymphocytic leukemia (CLL) cells, using the fluorometric microculture cytotoxicity assay (FMCA).[9] The FMCA is based on cellular hydrolysis of fluoresceindiacetate (FDA) to flourescent fluorescein by cells with an intact cell membrane. Briefly, patient samples were obtained by routine blood sampling and tumor cells were isolated by Ficoll-Paque (Kabi-Pharmacia, Uppsala, Sweden) density gradient centrifugation and resuspended in culture medium. The tumor cell suspension was seeded into 96-well microtiter plates (Nunc, Roskilde, Denmark), prepared with serially diluted drugs at five different concentrations and the plates were incubated for 72 h. After incubation, drugs and culture medium were washed away, and FDA was added to test, control and blank wells. After 30 min the generated fluorescense was measured in a Fluoroscan II (Labsystems Oy, Helsinki, Finland) and cell survival was calculated, defined as the flourescense in test wells in per cent of that in the control wells, with blank wells substracted. Each drug was tested on three to eight consecutive samples and the IC_{50} was determined from the dose–response curves obtained.

Quality criteria for a successful assay was more than 70% tumor cells in the cell preparation, a signal in control wells more than five times that in blank wells and a coefficient of variation less than 30% in control wells. Only successful assays were included in the study.

Clinical $C \times T$ data at MTD was collected from the literature (see Table 1 for references). A logarithmic transformation of parameters was used and statistical correlations were made by linear regression.

Table 1. Log $C \times T$ at MTD and the mean of the logarithms of the individual IC_{50} values for CLL cells *in vitro* (three to eight samples) for $C \times T$-dependent (type 1) and cell cycle phase-specific (type 2) cytotoxic drugs ($C \times T$ values are expressed in μg h/ml and IC_{50} values in μg/ml)

Drug	log $C \times T$ at MTD	Mean log IC_{50}	Reference
Type 1			
amsacrine	1.35	−1.09	10
bleomycin	0.70	1.35	10
carboplatin	1.99	1.86	11
chlorambucil	0.38	0.28	10
cisplatin	0.29	0.08	10
dacarbazin	1.49	2.08	10
daunorubicin	0.86	−1.30	10
doxorubicin	0.58	−0.98	10
epirubicin	0.23	−0.13	12
etoposide	2.19	0.04	10
idarubicin	−0.71	−1.15	13
melphalan	0.39	0.20	10
mitomycin	−0.44	−0.18	10
mitoxantrone	0.28	−1.47	14
streptozocin	1.86	2.42	15
Type 2			
5-azacytidine	0.19	0.32	10
cladribine	−0.78	−1.17	16
cytarabine	−0.22	−0.50	10
fludarabine	0.47	0.51	17
5-fluorouracil	1.21	1.83	10
gemcitabine	0.94	0.54	18
6-mercaptopurine	1.06	1.89	10
taxol	1.16	0.55	19
6-thioguanine	1.86	1.52	10
vinblastine	−0.76	−0.68	10
vincristine	−1.19	−1.82	10
vinorelbine	−0.10	−0.77	20

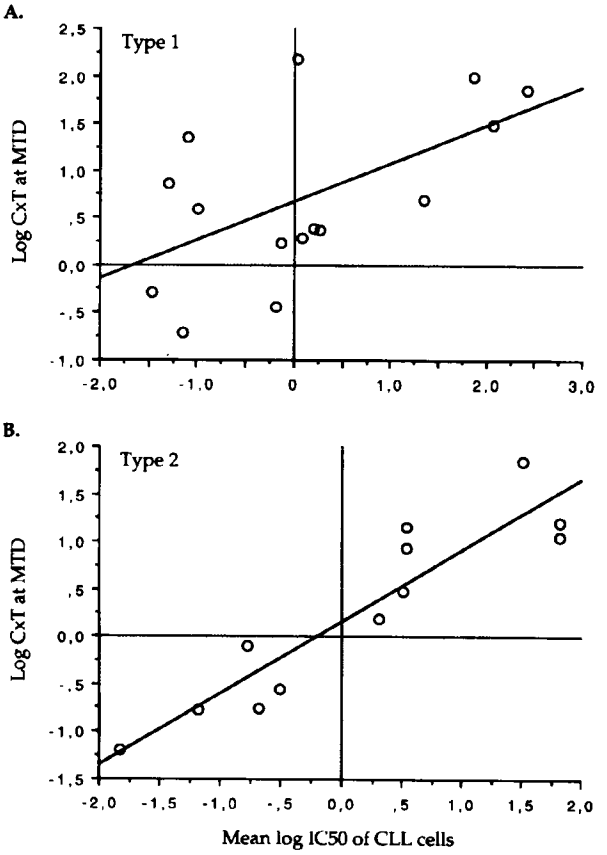

Figure 1. Linear regression lines for IC_{50} (μg/ml) of patient CLL cells versus clinical $C \times T$ (μg h/ml) at MTD of $C \times T$-dependent, or type 1 (A), and of cell cycle phase-specific, or type 2 (B), cytotoxic drugs. A logarithmic transformation of data was used. The correlation coefficients were 0.58 ($p = 0.024$) for type 1 drugs and 0.92 ($p < 0.0001$) for type 2 drugs.

Results and discussion

In Table 1 the logarithms of the clinical $C \times T$ and the mean of the logarithms of the IC_{50}s in primary cultures of CLL cells are listed for each of the 27 drugs tested. When linear regression analysis was performed on the two logarithmic parameters, a high and significant correlation was observed for the type 2 drugs ($R=0.92$, $p<0.0001$), while for the type 1 drugs the relationship was weaker ($R=0.58$, $p=0.024$). The regression lines are presented in Figure 1.

Fuse *et al.* confirmed that human $C \times T$ may be predicted from mouse $C \times T$, suggesting that PGDE is a useful method for determining dose escalation for $C \times T$-dependent (type 1) drugs. For cell cycle phase-specific (type 2) drugs, on the other hand, the correlation between mouse and human $C \times T$ was less impressive, and the authors suggested that PGDE should not be used in that group of drugs.[7] Interestingly, in the present study the opposite pattern was evident and the correlation between IC_{50} *in vitro* and clinical $C \times T$ was much better for type 2 drugs than for type 1 drugs. Similar correlations were obtained also by using the $C \times T$ data cited by Fuse *et al.* for the drugs common to both studies ($R=0.48$ for type 1 drugs, $n=6$ and $R=0.90$ for type 2 drugs, $n=7$). These results indicate that *in vitro* drug activity data on human hematologic cells may be a valuable complement to *in vivo* toxicology studies in mice when determining a safe starting dose and dose escalation procedure of type 2 drugs. Speculatively, if the ratio between the $C \times T$ predicted from mouse data and the $C \times T$ predicted from *in vitro* studies of a new drug is high, this may indicate pharmacodynamic species differences. Thus, a lower starting dose and careful dose escalation should be considered to avoid severe initial toxicity in humans. Conversely, if the ratio is near 1 or below, a PGDE protocol may be used with greater confidence, using $C \times T$ at LD_{10} in mice as target $C \times T$ (Figure 2).

The reason for the better correlation to $C \times T$ for type 2 drugs is not clear. Although continuous exposure may be required for optimal efficacy of type 2 drugs *in vitro*, the use of this exposure strategy cannot explain the poorer correlation observed for $C \times T$-dependent drugs. However, the relationship between pharmacokinetics *in vivo* and stability *in vitro* may be different for type 1 and type 2 drugs. Indeed, preliminary experiments relating *in vitro* stability under conditions of the FMCA to $C \times T$ at MTD *in vivo* for 11 type 1 drugs and six type 2 drugs showed a markedly better correlation between estimated $C \times T$ *in vitro* and $C \times T$ *in vivo* for the type 2 drugs ($R=0.87$ and 0.33 for type 2 and type 1 drugs, respectively; not shown) supporting this notion. However, further studies using a larger number of

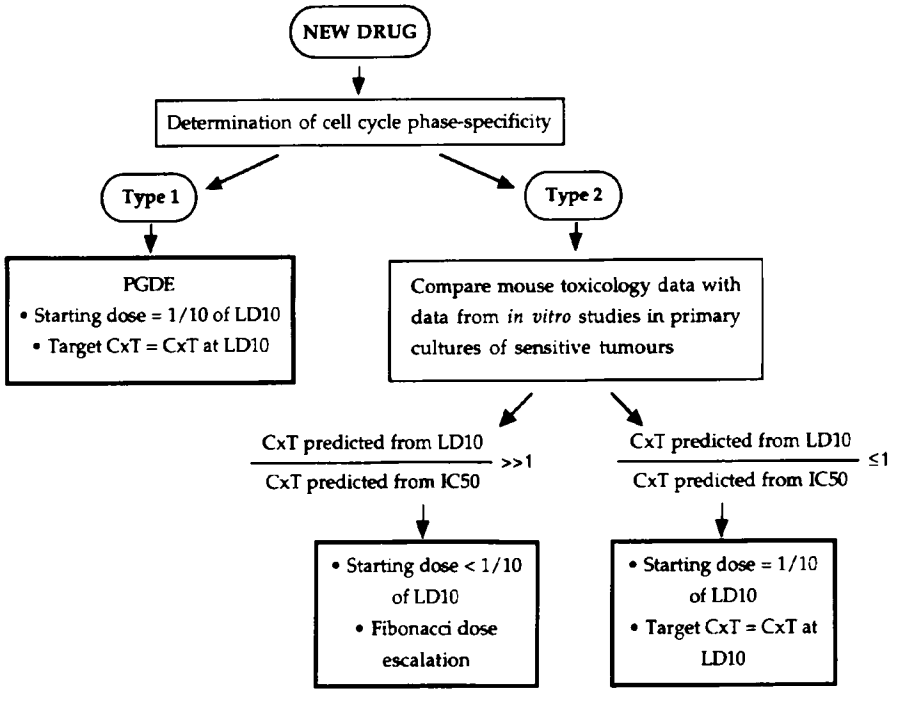

Figure 2. Hypothetical scheme for determining starting dose and dose escalation procedure in phase I studies of new cytotoxic drugs.

drugs are clearly needed to substantiate these findings.

As discussed previously,[8] there are some obvious limitations to the present result. The pharmacokinetic data for several drugs are relatively old, and protein binding and active metabolites were not considered. Also, a greater number of patient samples should be used for the IC_{50} determination since IC_{50} sometimes varies considerably between individual samples. Nevertheless, if these preliminary results are verified prospectively for a larger number of drugs using modern analytical techniques, and with IC_{50} data from more patients, *in vitro* cytotoxicity data obtained from human lymphatic cells may prove to be a helpful adjunct to animal toxicology studies and PGDE in the future.

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