The impact of pharmacokinetically guided dose escalation strategies in phase I clinical trials: Critical evaluation and recommendations for future studies

M. A. Graham & P. Workman

CRC Department Medical Oncology, University of Glasgow, CRC Beatson Laboratories, Bearsden, Glasgow, UK and EORTC Pharmacology and Molecular Mechanisms Group

Summary. Phase I studies requiring multiple dose escalation steps have led to the development of pharmacokinetically guided dose escalation (PGDE) strategies to expedite the conduct of early clinical trials. This article critically reviews PGDE strategies for a number of new anticancer agents including amphethinile, brequinar sodium, iodo-doxorubicin, the anthrapyrazoles (DuP 941, DuP 942 and DuP 937), rhizoxin, and aphidicolin glycinate. The benefits and problems associated with PGDE are examined. Recommendations are made for the optimal deployment of pharmacological information in future phase I studies.

Key words: pharmacokinetic strategies, phase I trials, dose escalation

Introduction

Over the last ten years, pharmacokinetic studies have been gaining increasing importance in the process of new anticancer drug development. Why is this? Undoubtedly the reason is that pharmacokinetic studies are widely perceived by both experimental and clinical oncologists as having a valuable role to play. In earlier days there was a tendency to regard pharmacokinetics as a dry science practised by aficionados who played their trade with HPLC equipment and computer models, presenting complex equations and incomprehensible tables of data, with scarce concern for the relevance to drug design and development. No doubt some felt it to be a necessary, but tedious or even eccentric occupation, pursued for its own sake or to satisfy the regulatory agencies. So what has changed?

We believe that two significant changes in attitude have taken place. The first is that those involved directly in pharmacokinetic studies have become much more proficient at integrating their activities into the drug development process – from the laboratory to the bedside. At the same time, clinicians carrying out early clinical studies have become convinced of the value of pharmacokinetic studies – indeed many of them have themselves been trained in the science of pharmacokinetics.

There are as yet very few examples where pharmacokinetic measurements are essential to the clinical practice of oncology. The gold standard is of course methotrexate, where monitoring of drug levels can avoid potentially fatal toxicity. There is however a rapidly developing interest in therapeutic drug monitoring – the individualization of drug dosage based on measured drug concentrations. Examples include etoposide and 5-fluorouracil for example [1]. The use of limited sampling strategies and Bayesian statistics will greatly help this approach. The successful development of simple dose individualisation formulae for carboplatin, based on renal clearance, also owes its success to a pharmacokinetic approach [2, 3].

Without a doubt the greatest impact of pharmacokinetics occurs further back in the drug development pipeline. Firstly, pharmacokinetic studies in animals are likely to be carried out at an earlier stage than hitherto – perhaps even before a lead compound emerges. Thus this information can feedback into the drug design process. Secondly, pharmacokinetic analysis is now carried out routinely to aid interpretation of toxicology studies. Thirdly, pharmacokinetics are essentially mandatory to the conduct of a modern phase I clinical trial. Most major cancer centres participating in phase I studies have access to appropriate analytical equipment and pharmacokinetic expertise.

It is in this general context – of a growing appreciation of the value of a knowledge of how the drug is handled by the body – that the concept of pharmacokinetically-guided dose escalation or PGDE has been developed.

Origins of PGDE

PGDE was originally proposed by Collins and coworkers [4] in the Blood Level Working Group of the Division of Cancer Treatment at the US NCI. The impetus came from the widespread frustration that many phase I studies were requiring an unacceptable number of dose escalations before the maximum tolerated dose (MTD) could be defined. For example a

phase I study of flavone acetic acid in this department required a total of 14 dose escalations [5]. What this means is that in such cases the MTD in man is being poorly predicted by animal toxicology studies. In general toxicological investigations are carried out in mice, rats and sometimes dogs [6,7]. Overall, the LD10 dose in mice, when expressed in units of surface area, is a fairly accurate predictor for the MTD in man [4, 6, 8–12]. However, in individual cases the human MTD can vary from one-tenth to ten times the mouse LD10. Because of the lower limit of one-tenth, the phase I starting dose is usually set at this fraction of the mouse LD10 in order to introduce an appropriate margin of safety without unduly restricting the initial doses to an unnecessarily homeopathic level. Again on average, about 5 dose escalations would be required to reach the MTD using the standard 'modified Fibonacci' scheme (sequential increments of 100%, 67%, 50%, 40% and then 30%–35%). Clearly, where the entry dose is lower than normal or where the mouse gives a falsely low prediction of the human tolerance, a greater number of escalations will be needed. This inevitably means that the phase I study will consume more resources, time and patients, and in addition decreases the likelihood that the patients might receive benefit from the drug (the objective response rate in phase I trials is 4% [13]).

Collins et al. [4] recognized that disparities in drug tolerance between species may be due to differences in systemic (i.e. plasma) pharmacokinetics and/or pharmacodynamics – the latter term referring to target cell sensitivity. They then sought to estimate the pharmacokinetic element by comparing retrospectively the drug exposures at the mouse LD10 and the human MTD. To do this they used the standard exposure parameter: area under the plasma concentration versus time curve (C×T or AUC). For several drugs the AUC values gave better agreement than did the dose, indicating that pharmacokinetic differences were particularly important. Doxorubicin was an especially good example of this, whereas with antimetabolites the AUC correlations were poor. In the latter case differences in intracellular handling and metabolism almost certainly predominate.

Impressed by these findings, Collins et al. [4] went on to suggest that comparative pharmacokinetic measurements in mice and men could be used to guide the phase I dose escalation. Consider the situation where the plasma AUC at the phase I entry dose is very small compared to that at the LD10 in the mouse: the solution is to escalate more aggressively, monitoring the AUC at each stage, until the human exposure approaches 40% of the mouse LD10 exposure. At that point the escalation can be completed by a modified Fibonacci method. Two possible approaches were suggested: one involved using a single PGDE step by a factor equal to the square root of the ratio of mouse LD10 to the human entry dose (the geometric mean method) while the other required a progressive doubling of the dose. The latter method, sometimes called the extended factor of two method, has received the widest acceptance, probably because of concerns over using dose escalations greater than a doubling. It should be mentioned that where the AUC at the phase I entry dose is close to the target (mouse LD10) AUC, then the escalation would actually proceed more cautiously than with the conventional approach.

An attractive feature of the PGDE concept is that it is rationally based. Moreover, although it should be a very safe method, it nevertheless permits a faster dose escalation in many cases. The retrospective study of selected drugs showed that the potential savings are large: 5–6 steps rather than twice that number with doxorubicin, for example.

Further development of PGDE

Stimulated by the results of the Blood Level Working Group, the EORTC Pharmacokinetics and Metabolism Group (now the EORTC Pharmacology and Molecular Mechanisms Group) in turn analyzed their considerable retrospective experience of pharmacokinetics in relation to toxicity [14]. Despite expressing certain concerns about the quality of the data in their retrospective analysis, it was felt that PGDE would have been of value in a number of cases. Particularly good examples were certain anthracyclines and platinum complexes. Details of the excellent retrospective study with 3 platinum compounds were published by van Hennick et al. [15]. In contrast the thorough study of Kerpel- Fronius et al. [16] showed that AUC considerations did not help with the explanation for the observation of a lower toxicity for the alkylating agent diacetyl dianhydrogalactitol in humans compared to mice. Chloroethylating agents such as nitrosoureas and temozolomide did not perform well. Not surprisingly, drugs requiring metabolic activation also gave poor correlations (e.g. melamines, dacarbazine, N-methyl formamide) as did the antifolate MZPES where peak plasma levels predicted better than AUC for the acute neurotoxic effect [14].

The EORTC PAM Group report emphasized that particular care should be taken where there are species differences in plasma protein binding or metabolism. Another important point is that the existence of nonlinear pharmacokinetics will generally preclude the use of the 'conventional' PGDE approaches mentioned above. On the other hand, pharmacokinetic monitoring is especially important in this situation because a small increment in dose may produce a disproportionally large increase in AUC. Potential technical difficulties such as route of administration, formulation and assay sensitivity were also highlighted. Inter-patient heterogeneity was also thought to be a potential problem.

Based on the two initial publications [4, 14] and associated studies, there was a strong feeling that PGDE should be subject to a thorough prospective analysis. In addition to North American investigators, the EORTC

New Drug Development and Coordinating Committee and the CRC Phase I/II Clinical Trials Committee have endorsed this view and are now testing the approach in their own trials. The main aim of the present paper is to review the prospective experience which has been published so far. For further background the reader is referred to the update by Collins et al. [17] and the commentary by Newell [18].

Basic recommendations for PGDE

Both Collins et al. [17] and the EORTC PAM Group [14] have emphasized that there should not be a standard rule book for PGDE. It is important to gear the approach to the pharmacology of the individual agent. Nevertheless some basic principles have emerged. The minimum requirements are:

1. Determine the plasma AUC at the mouse LD10, checking for non-linearity and also for protein binding in mouse and human plasma. The same batch of mice should be used for the toxicity and AUC measurements and the clinical route and formulation should normally be employed.
2. Initiate the phase I study at one-tenth the mouse LD10 dose and measure the plasma AUC.
3. Dose escalate to the MTD as appropriate, monitoring the AUC at each stage. In general this will be done by doubling the dose to approach 40% of the mouse LD10 (target) AUC, completing by a conventional escalation.

The more extensive requirements proposed by the EORTC PAM Group [14] are:

1. Determine the presence of metabolites in mice and their contribution to the drug's effects.
2. Develop an assay for the drug (and metabolites) sensitive to one-tenth of the mouse LD10 dose.
3. Determine the mouse LD10 with acceptable confidence limits and then measure the AUC for the drug (and active/toxic metabolites) at the LD10, half the LD10 and one-tenth the LD10. Watch out for non-linear kinetics.
4. Quantitate protein binding in mouse and human plasma at relevant concentrations.
5. Initiate the clinical study at one-tenth the mouse LD10 dose and treat 3–5 patients to determine the AUC with acceptable accuracy.
6. Use an appropriate escalation scheme to reach the target AUC with monitoring of drug and metabolite levels at each escalation and modify as required for non-linearity.

Review of the prospective PGDE experience

Amphethinile

The spindle poison amphethinile was one of the first agents to prospectively undergo PGDE in a phase I study. Preclinical toxicological studies estimated that the acute i.v. LD10 was 400–411 mg/m^2 from a which a phase I starting dose of 40 mg/m^2 was derived (Table 1). The pharmacokinetics of the drug were investigated in mice at 100, 200 and 400 mg/m^2 to establish a target AUC (313 μg l^{-1} h) and to check for linear pharmacokinetics [19]. These preclinical analyses revealed that the pharmacokinetics of amphethinile were non-linear in mice as each doubling of the dose resulted in a 3–4-fold increase in AUC [19]. Despite non-linear pharmacokinetics it was decided to attempt a PGDE strategy in the phase I with the intent of escalating the dose by a modification of the geometric mean method [4]. Included in these proposals was the provision for a maximal initial dose escalation of 5n (where n is the phase I starting dose) providing that the AUC at the starting dose was <5% of the target AUC [19]. Phase I studies were initiated at one-tenth the mouse LD10 (40 mg/m^2) and pharmacokinetic monitoring performed in 3 patients. Unfortunately the drug could not be detected at the starting dose, a major contributory factor being the relative insensitivity of the assay method employed (100 ng/ml) [19]. On the basis of the failure to detect the drug at 40 mg/m^2 the dose was escalated by a large increment (5n) to 200 mg/m^2 at which the pharmacokinetics of the drug could be estimated. In retrospect this aggressive dose escalation strategy is questionable, particularly in view of the non-linear pharmacokinetics of amphethinile in mice. However, no serious toxicity was encountered in the 3 patients treated at this dose level with the exception of one patient who experienced a grand mal convulsion following a second course [19]. It was felt that this toxicity was probably related to the rate of injection, a phenomenon which had been observed preclinically in mice. Subsequently, the dose was progressively doubled to 400 and 800 mg/m^2, but given as a short infusion rather than a bolus injection to alleviate acute neurotoxicity. Although the AUC values at 800 mg/m^2 ranged from 24–81 μg l^{-1} h, considering that dose limiting toxcities were absent, the dose was escalated further to 1200 mg/m^2. Severe nausea/vomiting and alopecia were experienced and two deaths occurred within 48 h after drug treatment at 1200 mg/m^2, at which point the trial was terminated. Pharmacokinetic analysis at this last dose level revealed that the plasma AUC values (361 μg l^{-1} h) were either similar to the target AUC (313 μg l^{-1} h) or substantially lower (154–195 μg l^{-1} h) (Table 1). Surprisingly, the lower values were associated with the fatalities.

The experience with amphethinile illustrates some of the difficulties with implementing PGDE studies. It stresses the importance of developing sensitive analytical procedures to detect the drug at the starting dose and highlights the problems of non-linear pharmacokinetics. In retrospect, employing a smaller initial dose increment (2n) may have been more appropriate, although no serious toxicities were encountered following the 5n dose escalation. However, this may not be

Table I. Prospective evaluation of pharmacokinetically guided dose escalation strategies in phase I trials.

Drug	Class and schedule	Mouse LD10 (mg/m²)	LD10 AUC	Target AUC	Starting dose (mg/m²)	Human MTD (mg/m²)	Mean AUC at MTD	Protein binding (%)		Comments	Refs
								Mice	Human		
Amphethinile	Spindle poison (once every 3 weeks)	411	313[a]	313[a]	40	1,200	236[a]	ND	ND	Limited assay sensitivity. Drug not detectable at starting dose. Escalation accelerated.	19
Brequinar sodium	Antimetabolite (once every 3 weeks)	396	10,070[b]	10,070[b]	15[b]	2,250	7854[b]	ND	ND	Combination of modified Fibonacci and PGDE when mouse data became available. Escalation accelerated.	20
Iodo-doxo-rubicin	Anthracycline (once every 3 weeks)	19	5[c]	5[c]	2	80	0.31[c]	96	93	Marked species differences in metabolism to I-Doxol. I-Dox and I-Doxol AUCs were summed and doses escalated by PGDE scheme using original I-Dox target AUC. Escalation accelerated.	21 23 24
I-Doxol	Metabolite	20	6.8[c]	NA	NA	NA	4.04[c]	95	92		
DUP-941	Anthrapyrazole (once every 3 weeks)	52	277[d]	110[d]	5	50	288[d]	84	95	Wide interpatient variability in drug clearance at starting dose PGDE scheme could not be used.	28 30 31
DUP-941	(once weekly for 3 weeks)	ND	ND	277[d]	2	24	151–399[d]	84	95	The target AUC in this study was based on the AUC with a single i.v dose LD10 [28]. Drug was not detected at the starting dose and wide interpatient variability in clearance prevented the use of a PGDE scheme.	28 32
DUP-942	Anthrapyrazole (once every 3 weeks)	75	177[e]	59[e,i]	7.5	160	126[e]	ND	ND	Escalation accelerated using a PGDE scheme (6–9 fewer patients claimed).	35
DUP-942	(once every 3 weeks)	75	300[f]	120[f]	7.5	150	435[f]	ND	ND	PGDE could not be used due to assay insensitivity at starting dose and rapid plasma clearance. AUC at human MTD exceeded LD10 AUC by 40%	36
DUP-937	Anthrapyrazole (once every 3 weeks)	36	3145[g]	1258[g]	3.6	25.2	5271[g]	ND	ND	PGDE could not be used due to wide inter-patient variability in drug clearance.	37
Rhizoxin	Spindle Poison (once every 3 weeks)	8–12	71[d]	28[d]	0.8	2.6	0.45[d]	96	97	Drug not detectable at starting dose. Marked species differences in plasma AUC values. Suspected pharmacodynamic differences in bone marrow stem cell sensitivity. PGDE scheme could not be used.	39
Aphidicolin glycinate	DNA polymerase inhibitor (once daily for 5 days every 3 weeks)	300	40.1[h]	16–40[h]	12	2250	62.5	ND	ND	Pharmacokinetics assisted dose escalation. AUC predicted better than dose. MTD from daily × 5 study increased the entry dose for the 24 hour continuous infusion schedule.	40
	(24 hour continuous infusion)	ND	ND	ND	435	4500	157	ND	ND		

[a] μg l⁻¹ h⁻¹; [b] μg ml⁻¹ h; [c] μM h; [d] μM min; [e] μg ml⁻¹ min; [f] μmol l⁻¹ min; [g] ng ml⁻¹ h; [h] Starting dose = 1/4 toxic dose low in the dog;
[i] AUC values determined at mouse LD50 (90 mg/m²). Target AUC calculated as follows: 177 μg ml⁻¹ min × 75/90 × 0.4 = 59 μg ml⁻¹ min

the case for other anti-tumour agents and it is recommended that dose increments do not exceed a factor of 2n in the absence of pharmacokinetic data at the starting dose or if non-linear pharmacokinetics are suspected.

Brequinar sodium

Brequinar sodium is a novel quinoline carboxylic acid analogue which blocks de novo pyrimidine biosynthesis by inhibiting mitochondrial dihydroorotate dehydro-

genase. As a prelude to clinical studies, the toxicity of brequinar sodium was evaluated in mice following a single i.v. bolus dose and the LD10 estimated as 396 mg/m^2 [20]. A check on the toxicity of the proposed phase I starting dose in a second species, the dog, proved toxic. Hence a starting dose equivalent to one-third of the toxic dose low (TDL) in the dog, namely 15 mg/m^2, was derived for phase I investigations (Table 1). At the onset of the phase I study no pharmacokinetic or target AUC data were available and for the first half of the trial dose escalations followed a modified Fibonacci scheme up to 300 mg/m^2 without encountering any severe toxcity [20]. Mouse pharmacokinetic data (determined at the LD10 dose only) became available during the trial, and revealed that the plasma levels at 300 mg/m^2 were approximately 1/20th the target AUC. On the basis of these data and the lack of any measurable toxicity, the dose was doubled for the next two steps up to 1200 mg/m^2. Subsequent escalations were based on clinical judgement with 25% incremental rises in poor risk patients and 50% increments in good risk patients until the MTD had been established. This study can be acclaimed as a successful example of a PGDE strategy which saved at least three unnecessary dose escalation steps, as compared to a conventional modified Fibonacci approach [20].

Iodo-doxorubicin

4′-Iodo-4′-deoxydoxorubicin (I-Dox) is one of a number of new anthracycline derivatives, substituted in the daunosamine sugar, which possesses anti-tumour activity in anthracycline resisitant tumours and reduced cardiotoxicity in experimental models [21, 22]. In a prospective phase I study a PGDE strategy was attempted on the basis of somewhat limited mouse pharmacokinetic and toxicity data [23]. A particularly thorough clinical study was documented by Gianni et al. [24] which incorporated the essential components of the proposals by Collins et al. [4] and the refinements of the EORTC Pharmacokinetics and Metabolism Group [14]. A detailed and well conducted strategy was devised to overcome problems encountered as a result of marked differences in the metabolism of I-Dox in mouse and man [24].

Clinical testing of I-Dox commenced 2 mg/m^2, approximately 1/10th of the mouse LD10 (19 mg/m^2), with the intent of escalating the dose to 40% of the target AUC (5 μM h) by an extended factor of two method [24]. However, at the starting dose it became apparent that there were marked species differences in the pharmacokinetics and metabolism of the drug. This disparity was principally due to the rapid and extensive formation of 4′-iodo-4′-deoxy-13-dihydrodoxorubicin (I-Doxol) due to an aldo-keto reductase which was absent in mice [24]. Consequently, the initial 6 dose escalations up to 26 mg/m^2 utilised a modified Fibonacci scheme whilst the pharmacology of I-Dox and I-Doxol were investigated further. A particularly commendable aspect of this study was the series of comparative pharmacological investigations on I-Dox and I-Doxol in vitro and in vivo. Firstly, this study demonstrated the equivalent toxicity of I-Dox (IC50 50nM) and I-Doxol (IC50 80nM) against the growth of human bone marrow cells in vitro (CFU-GM assay). Secondly, the toxicity of the parent drug and metabolite were shown to have comparable LD10 values in mice, namely 6 mg/kg and 6.8 mg/kg for I-Dox and I-Doxol respectively (Table 1) [24]. As a result of these findings a PGDE scheme was re-introduced and subsequent doses escalated by the summation of the I-Dox and I-Doxol AUCs, utilising the original target I-Dox AUC to guide the dose increments. At 26 mg/m^2 the mean sum of these AUCs was 1.4 μM h which was 24% of the target AUC. It was at this point that the authors then abandoned the Fibonacci scheme, for which escalations were already limited to 35%, and doubled the dose of 52 mg/m^2. At this dose grade 4 granulocytopaenia was seen in one patient and the Fibonacci escalation was therefore resumed to complete the study. Pharmacokinetic analysis at the MTD of 80 mg/m^2 revealed a close match between the AUC at the mouse LD10 and sum of the I-Dox/I-Doxol plasma AUC in patients, which lends support to the original Collins hypothesis [4]. Use of PGDE at a relatively late stage saved a single dose escalation step. What is particularly encouraging from this study is the notion that the MTD could have been reached in only five steps had the comparative pharmacological information on I-Dox and I-Doxol been available at the outset.

Anthrapyrazole DuP941 (CI-941)

The anthrapyrazoles were synthesised in an attempt to find a non-cardiotoxic DNA binding drug which retained or possessed superior anti-tumour activity to doxorubicin [25–27]. Three lead compounds were identified (CI-941, CI-942 and CI-937 now prefixed DuP), each of which displayed excellent anti-tumour activity against murine tumours [26] all three were subsequently developed for phase I clinical testing. PGDE strategies were developed for all three agents but these met with mixed success due to a number of unanticipated problems.

It was recognised from the experience with amphethinile that sensitive analytical methods were a prerequisite for PGDE studies. A highly sensitive solid phase extraction and HPLC assay (limit of detection 1 ng/ml) was developed for DuP941 which was subsequently used to describe the pharmacokinetics of DuP941 in mouse and human plasma [29]. The preclinical pharmacokinetic and toxicity studies of DuP941 were integrated so that direct correlations could be drawn between drug exposure (AUC) and toxicity (i.e. LD10/LD50). These studies were performed in the same batch of randomised animals, with the clinical formulation of the drug, given by the same route of administration to be used clinically (i.v.) [28].

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