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One Step Closer to the Target: Intracellular Pharmacokinetics of Gemcitabine

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1. Introduction

Drug dosing is based on the premise that the amount of drug given influences the toxicity and response. The premise of pharmacokinetics is that it is not the amount of drug given, but the amount the patient is exposed to, that determines drug effect. Measurements of drug exposure are usually performed by determining the concentration in serum or plasma. This accounts for variability in drug clearance in determining the patient's exposure. This has resulted in such dosing strategies as carboplatin dosing based on creatinine clearance, therapeutic dose monitoring of methotrexate and pharmacogenetically modified dosing for irinotecan.

However it is well recognised that the plasma is not the compartment that is most relevant in determining tissue effects. For example for the antibiotic azithromycin the organ concentrations in the site of infection, such as lung are the critical feature in determining drug effect for pneumonia (Olsen et al., 1996; Schentag & Ballow, 1991). This argument has been extended to cancer anti-metabolites (Shewach & Lawrence, 2003).

The situation in the treatment of a patient with cancer is even more complex. Tumours occur in an organ of interest such as the lung, or brain and liver. Regional concentrations in these organs are important variables (Reid, et al 1990; Breedveld et al., 2005). However tumours also create their own microenvironment with alterations in blood supply, interstitial pressure and local secretion of growth factors and cytokines (Liotta & Kohn, 2001; Wiig, et al, 2010). The drug concentration of interest is the concentration at the tumour site. Differences in tissue PK (C. Presant et al., 1990) and inter-tumoural and intra-tumoural PK are additional considerations (Zamboni et al., 2002). Therefore variation in the pharmacokinetics of plasma is only one source of variation between patients.

The reason why plasma is sampled is, of course because it is easily accessible. There are limited possibilities for repeated tumour biopsies, although this has been done (Eisbruch et al., 2001) and although there are some techniques for sampling closer to the tumour such as microdialysis (Jain, 1987), nuclear magnetic resonance spectroscopy (Müller et al., 1997) and positron emission tomography (Gupta et al 2002) these have had limited application. Nevertheless it is important to recognise the limitations of plasma and aim for sampling closer to the target where possible.

A special case is that of intra-cellularly activated drugs. The most important class of these drugs in oncology are the nucleoside analogues such as ara-C, fludarabine and gemcitabine. In this situation the drug administered is a pro-drug, which is activated intracellularly. Because the enzymatic pathways required for drug activation are well characterised there is therefore an excellent opportunity to move the consideration of drug pharmacokinetics one step closer to the target, by considering intra-cellular pharmacokinetics.

The pharmacogenetics of gemcitabine are summarized with a curated bibliography at PharmGKB. The reader is also referred to previous reviews for more detailed reports (Danesi et al., 2009; Galmirini et al., 2001; Nakano et al., 2007; Veltkamp et al., 2008). The purpose of this chapter is to bring together the data on pharmacokinetics, pharmacogentics, pharmacodynamics and associated models to provide a worked example of the potential for integrating this information and the utility and limitation of moving one step closer to the target.

2. Gemcitabine background

Gemcitabine (GEM) is a deoxcytidine analogue (2′,2′-Difluorodeoxycytidine) which mimics the normal actions of the pyrimidine nucleoside deoxycytidine in its transport, phosphorylation to the tri-phosphate and then incorporation into DNA. Its structure is shown in Figure 1. However during DNA synthesis only one further base can be added then the DNA polymerase is unable to pass the altered base and DNA chain termination results. This causes an S-phase specific cell cycle arrest and programmed cell death (P. Huang, et al, 1991).

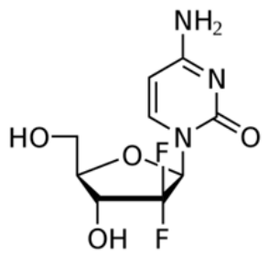

Fig. 1. Chemical structure of Gemcitabine (Creative commons license Wikimedia commons)

The enzymes responsible for each step of these processes are well characterised and variation in their activity are potential causes of variation in the response to GEM. Details on each gene of interest are provided on the entrez-gene link provided. Understanding which steps are causes of variation in toxicity and response and how to model the effects of multiple variables are key issues in understanding this variation. This requires understanding the relationship between the pharmacokinetics of GEM in the plasma and the intracellular activity of gemcitabine triphosphate (GEM-TP). The pathway of GEM activation and inactivation is illustrated in Figure 2.

The importance of understanding this is underlined by the fact that GEM is a commonly used drug in a wide range of important cancer types including non-small cell lung, breast, pancreatico-biliary and bladder.

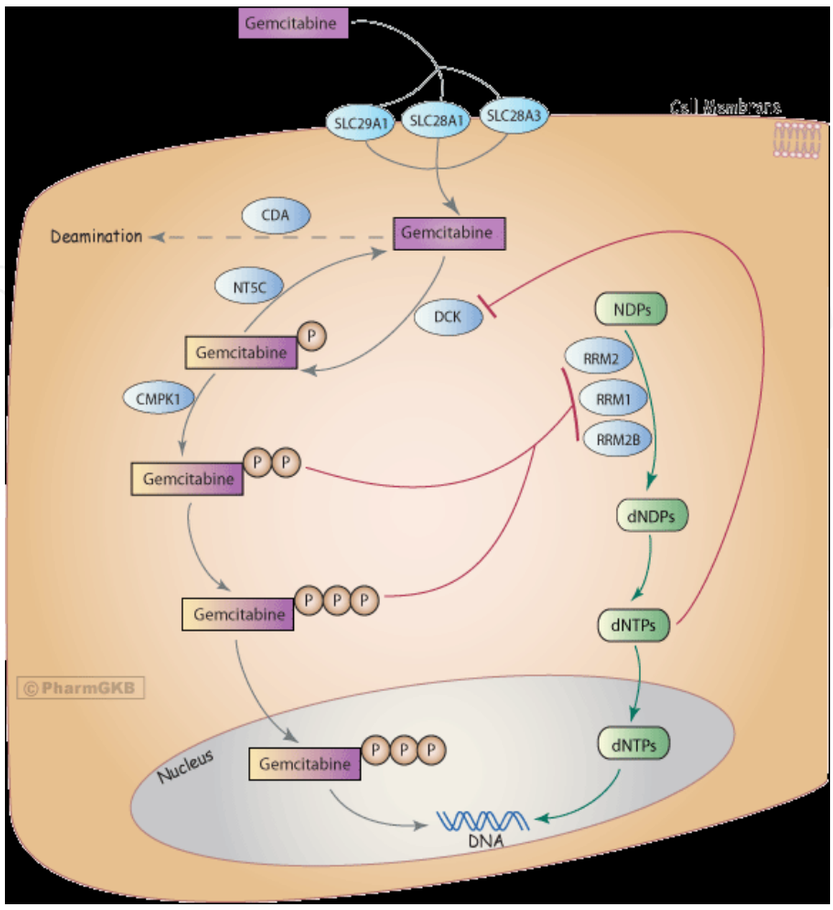

Fig. 2. Gemcitabine pathway (copyright PharmGKB, permission has been given by PharmGKB and Stanford University)

2.1 Uptake

The first step in activation is an active process of transport into the cell. The main mechanism of uptake is via hENT1 (SLC289A1) (Mackey et al., 1998) although a smaller portion is carried via other transporters SLC28A1 and SLC28A3 (Graham et al., 2000).

2.2 Phosphorlyation

Intracellular retention requires phsophorylation via the kinases, deoxyctidine kinase (*DCK*) and *CMPK1*. The rate- limiting step in this process is DCK. Phosphorlyation promotes intracellular retention and allows incorporation of GEM-TP into DNA.

2.3 Inactivation

The major route of inactivation is via deamination via Cytidine deaminase(CDA). *CDA* is ubiquitously expressed and converts GEM to difluorodeoxyuridine (dFdU). dFdU has low level activity and is water soluble and excreted almost entirely by renal excretion. A minor route of deactivation is through dephosphorylation of GEM-TP via 5'Nucleotidase (Dumontet et al., 1999).

2.4 Self- potentiation

One of the remarkable aspects of GEM pharmacology is that it has multiple mechanisms of self-potentiation (W Plunkett et al, 1996). The most important mechanism is that GEM-TP and GEM-DP block the salvage pathway of nucleotide synthesis through inhibition of Ribonucleotide reductase. This has the effect of reducing the pool of nucleotides that are competing with GEM-TP for incorporation and thereby increasing GEM-TP incorporation into DNA. Ribonucelotide reductase is composed of 2 subunits RRM1 and RRM2. The importance of this pathway is underlined by the data that variation in RRM1 is a critical determinant of GEM response (see later).

A second mechanism of self-potentiation occurs via inhibition by GEM of dCMP deaminase, which results in a reduced catabolism for GEM-TP (Heinemann et al., 1992) and a third mechanism of self-potentiation is that the main metabolite of GEM, dFdU (Hodge, et al,, 2011) increases GEM-TP accumulation in a time dependent manner.

3. Pharmacogenetics of GEM

There is considerable inter-patient variability in gemcitabine accumulation and there are polymorphisms described in many of the genes involved in transport, activation and inactivation. Data on pharmacogenetics for key drugs is agregated on the PharmGKB wesite (http://www.pharmgkb.org) and gemcitabine is included in this collection. An excellent review has been published by Wong et al (2009).

3.1 Understanding the significance of polymorphisms

Understanding the significance of a polymorphism will depend upon its changes in structure, functional significance and the frequency of variation. Non coding SNP's can be associated by chance, by linkage disequilibrium or potentially through influences on , alternate splicing, microRNA binding or DNA morphology. Changes in the promoter region can lead to variation in expression. The significance depends more fundamentally on the impact that the coded protein has on function. There are two issues here –how much does each gene product impact on function and how much of human variation is accountable by variation in this gene.

For example there are 23 snp's in the gene SLC28A3 in the dbnsnp database http://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?locusId=64078 . Of these most are very infrequent with the heterozygozity rate of 15 loci are > 1% and only 6 are > 5%. Of the six most frequent SNP's, 3 are synonymous. These considerations are complicated by the issue that infrequent polymorphisms with major effects on function can have severe consequences.

Understanding the functional importance of a hapoltype or SNP ultimately requires demonstration of an effect on toxicity or efficacy. Pharmacokinetics however plays a crucial intermediate role in mediating pre-target variation. An association between a genetic variation and pharmacokinetics of the drug greatly increases the pre-test probability that functional differences will be found that are not related to chance.

3.2 CDA

A summary of SNP's in CDA is found in the geneview of the dbDNP database.

In European populations CDA:79A>C (K27Q) where the C allele is seen with a frequency of 0.36. Patients with a copy of the variant allele have reduced clearance and increased haematological toxicity. In Japanese patients this SNP is uncommon but a less common variant G208A has been associated with markedly reduced clearance and increased toxicity. The comparison between the two SNP's shows the importance of both common variations (in contribution to interpatient variability) and uncommon but functionally very important variants in contributing to toxicity "outliers".

3.3 RRM1

A summary of SNP's in RRM1 is found in the gene view of the dbSNP database. RRM1 is of particular interest because there is strong data to support the influence of mRNA protein expression on survival in patients with NSCLC treated with gemcitabine (Ceppi et al., 2006)(Rosell et al., 2004). The data supporting a relationship between RRM1 polymorphisms is not as strong. There are two relatively common polymorphism in the European population c.2223A>G and 2464G>A with minor allele frequencies of 0.5 and 0.16 respectively. The 2464G>A has been shown to be associated with GEM sensitivity in vitro (Kwon et al., 2006)and haplotype is associated with response (but not survival) in patients with NSCLC treated with GEM (Kim et al., 2008). A small study suggested a relationship between -37C>A and progression free survival (PFS) (S. Dong et al., 2010).

3.4 hENT1

In patients with pancreatic cancer treated with gemcitabine hENT1 transcript levels were associated with survival (Giovannetti et al., 2006). However identified polymorphisms are infrequent (SNP:Geneview) and 201A>G has been identified with increased neutropenia (Tanaka et al., 2010). Sugiyama found no relationship with gemcitabine pharmacokinetics (Sugiyama et al., 2001)

Polymorphisms of other potentially important genes such as other transporters and DCK have not yet been shown to have clinical importance.

3.5 Haplotypes

There is a growing view that polymorphism in single genes are unlikely to be informative and that more relevant information is likely to come from sets of informative genes (haplotypes)(Kalow, 2006) or more complex genetic variations (Lee & Morton, 2008). Because of linkage disequilibrium, some combinations will occur more frequently than others and it is therefore possible to map the most common gene combinations and test their significance.

This approach has been taken with CDA in the Japanese population where one haplotype (of various CDA SNP's) termed *3 was associated with reduced clearance of gemcitabine and increased toxicity (Sugiyama et al., January 2010).

Tanaka et al have taken a comprehensive approach (Tanaka et al., 2010; Okazaki et al, 2010). They first screened 17 polymorphisms in relevant (CDA, *dCK, RRM1, hCNT2, hCNT3*, and *hENT1*) genes in a sample of 149 patients with pancreatic cancer receiving chemo-radiation and identified polymorphisms with significant associations for toxicity or trends for effects on survival. None of the SNP's singly was associated significantly with survival.

They then went on to examine the relationship between SNP's and identified that two or three SNPs each of the *dCK, RRM1, hCNT2, hCNT3*, and *hENT1* (IVS12-201A>G and IVS2-549T>C) genes were in linkage disequilibrium ($|D'|>0.5$, $P < 0.01$). This enabled identification of relevant haplotypes.

They next examined relationships between haplotypes and overall survival (OS). The *CDA* C111T and A-79C TA haplotype was significantly associated with increased risk and the *hENT1* A-201G, T-549C, and C913T ACT haplotype was significantly associated with reduced risk of death compared with the most common haplotype of each gene respectively ($P < 0.05$).

They were also able to examine the effects of gene dose by comparing outcomes according to the number of poor risk genotypes. Patients carrying 0 to 1 ($n = 43$), 2 to 3 ($n = 77$) or 4 to 6 ($n = 30$) variant alleles had a Median Survival time T of 31.5, 21.4, and 17.5 mo, respectively.

This work shows the need to consider multiple significant genes in determining significance and the added power this analysis brings. However as discussed below there are fundamental limitations to the SNP approach, that relate to the relationship between genotype and phenotype.

3.6 Considering the influence of transcription and translation

One of the limitations of focusing on genetic polymorphisms as causes of functional variation is the potential influence of gene silencing (Meister & Tuschl, 2004) transcriptional (Goth-Goldstein et al, 2000) and post transcriptional regulation on protein expression (Cress & Seto, 2000). DNA sequence is only one determinant of ultimate function. Genes can be silenced by methylation or acetylation, alternate splicing of gene transcripts can lead to differences in function and allelic imbalance can lead to increased expression. Environmental influences on transcription such as inhibition or induction by drugs or endogenous messages are critical. One way of accounting for these variations is to concentrate on measurement of phenotype rather than genotype (Mahgoub et al, 1977).

With CDA phenotype has been assessed ex vivo by incubation of mononuclear cells, plasma or red cell extracts with pyrimidine analogues and measurement of deamination.

Similarly a furthers study found phenotype as assessed by in vitro deamination correlated with toxicity whereas specific polymorphisms did not (Ciccolini et al., 2010; Mercier et al, 2007)

4. Challenges in measurement of intracellular PK

There are a number of challenges in the measurement of both GEM and the intracellular metabolite GEM-TP. The first of these is related to the fact that CDA is present in plasma, therefore every blood collection tubes requires the presence of tetrahydrouridine (THU) to inhibit CDA. If THU is not included all of the GEM will be converted to dFdU its inactive metabolite in plasma before it can even enter the cell to be converted to the active metabolite GEM-TP. The next challenge is the fact that white cells cannot be stored frozen without the loss of triphosphates, therefore the triphosphates need to be extracted from leucocytes before any of the sample is frozen. This is not a complex processes but does require time and some experience is necessary thus can not easily be performed by a nurse that generally

collects the blood samples and adds to the complexity of the studies and probably the main reason why more intracellular measurements are not routinely conducted. The amount of leucocytes present in an individual blood sample is highly variable and can be effected markedly when patients are on chemotherapy. While the analysis of GEM and dFdU in plasma are relatively straight forward and can be quality controlled and give reliable results, the same cannot be said about the assay used to measure GEM-TP. This involves the use of an ion exchange column and high concentrations of salt to remove the triphosphate from the column. This makes the assay difficult to convert to more sensitive LCMS assays. It is not easy to quality control this assay which could lead to varying data being reported by different laboratoties.

5. Pharmacokinetics and dose optimisation

The pharmacokinetics of plasma Gemcitabine and its main metabolite have been described in multiple studies. The findings of the original phase 1 remain typical (JL Abbruzzese, et al., 1991). 77% of Gemcitabine was metabolised to dFdU and excreted in the urine in the first 24hrs dFdU clearance was proportional to creatinine clearance. The clearance of Gemcitabine in the plasma was rapid with a T1/2 of 8 mins. The clearance of GEM was independent of dose. The pharmacokinetics of GEM in some representative studies are shown in Table 1.

	Clearance (L/h/m²)	T1/2 min	AUC(µM*min)	Vss (L)	CMax(µM)
Soo et al., 2006					
Arm A (n29) FDR	164.0±64.0	18.2±4.2	1,346 ±1113	65 ± 37	21 ± 17
Arm B (n=29) 30 min	181.6±74.5	17.1±3.1	1,432 ±529	75 ±41	41 ± 14
Tempero, et al., 2003					
FDR					26 ± 6
Standard (higher dose)					100 ± 19
Grimison, et al 2007					
FDR n=31			1279±629		19± 6
Standard n=30			1642 ± 796		50± 18

Table 1. Representative Plasma Pharmacokinetic Data

The major clinical determinants of clearance are size and gender. The influence of size has been demonstrated through a correlation with BSA, which justifies the use of BSA to adjust dose. The influence of gender has been more variable although some studies show reduced clearance in women.

There is much less data on the pharmacokinetics of GEM-TP. Data from some representative studies are shown in Table 2. In the early development of Gemcitabine it was identified that

exposed cells demonstrate saturable accumulation of the Triphosphate. This correlates with the biochemistry of accumulation and retention being limited by intracellular conversion to the Triphosphate by DCK. In phase 1 studies this translated to a saturation of Gemcitabine accumulation in mononuclear cells with increasing dose. This is accompanied by a change in the pattern of elimination with monophasic elimination at low concentrations and biphasic elimination described after the threshold has been reached.

	Clearance (L/h/m²)	AUC(µM*min)	CMax(µM)
Soo et al., 2006			
Arm A (n29) FDR	5.2±2.0	35 079±18 216	174 ± 77
Arm B (n=29) 30 min	7.0±2.61	32 249±11 267	225 ± 74
(Tempero, et al., 2003)			
FDR			398
Standard (higher dose)			188
(Grimison, et al 2007)			
FDR n=31		2600 ± 2483	
Standard n=30		1833± 1833	

Table 2. Representative Intracellular Pharmacokinetic Data

The clinical correlate of this finding is that accumulation can be maximised by prolonging infusion time and indeed this is the case. Based on accumulation in leukemic blasts a target concentration and rate of administration was determined to maximise accumulation. There are several problems with this process. The target concentration and optimal rate of infusion has not been determined in solid tumours, where the kinetics are known to be different. Secondly the "optimum" rate of administration does not take into account inter-individual variation. Differential expression of DCK between tumours and normal tissue and between individuals would be expected to determine different optimum rates of administration.

The rationale for prolonged dosing received a major boost when a randomised phase 2 study demonstrated proof of principle that in a clinically relevant scenario (pancreatic cancer) prolonged infusion at a rate of 10 mg/m2/min (compared to a standard 30 min infusion) was associated with not only increased accumulation of GEM-TP, but with a trend for increased survival. This lead to a proliferation of similar studies in other tumour types confirming the pharmacokinetic finding but underpowered to demonstrate survival differences. Unfortunately a large phase 3 study, in pancreatic cancer, demonstrated that the pharmacological advantage obtained failed to translate into a significant survival advantage.

The challenges in optimising accumulation of Gemcitabine have been highlighted by data from our group showing an increase in Gemcitabine accumulation between week 1 and 2 with weekly administration (Grimison, et al., 2007). This suggests auto induction of Gemcitabine accumulation. In *vivo* modelling suggests this is a generic response to DNA damaging agents (Metharom, et al, 2010). The significance of this increase is not certain, with one study showing a non significant increase (de Lange et al., 2005), but it potentially complicates strategies to achieve a target intracellular concentration if the target changes over time.

We have furthered examined potential explanations for the discordance between the pharmacological advantage seen previously and the negative phase 3 study. Analysis of SNP's from our study has suggested that the pharmacological advantage of prolonged dose rate Gemcitabine is restricted to patients with the variant allele of c.CDA79A>C (Metharom et al., 2011). If this finding is confirmed, then the phase 3 study would have been underpowered to detect a survival difference in this population and consideration of the optimum dosing schedule may require individualisation according to genotype.

5.1 Pharmacokinetic/Pharmacodynamic relationships

The availability of pharmacokinetic data for both the prodrug and the active intracellular metabolite leads to the possibility of exploring differences in the ability of these parameters to predict drug effects of toxicity and efficacy.

There is data that demonstrates correlations between the concentration of Gemcitabine at the end of the infusion and haematological toxicity, however the relationship is weak. There is very limited data exploring the relationship between the pharmacokinetics of intracellular GEM-TP and toxicity. No relationship was seen in combination with Carboplatin (Soo et al., 2006) In our data GEM-TP cMax correlate with AUC and was used for pharmacodynamic correlations. Modelling GEM-TP concentrations to % reduction in white cells improved the fit of the model although the relationship remained modest (r^2 = 0.3739) (Grimison et al., 2007). This provides proof of concept that moving from the plasma to intracellular compartment did improve predictive ability, but that the proportion of variance related to pharmacodynamic variability and the relationship of the sampled compartment to the compartment of interest will determine the utility of this approach.

The relationship of pharmacokinetics to tumour response is much more difficult to assess as tumour Reponses are heterogeneous and seen in a minority of patients treated with Gemcitabine. This then requires PK sampling in large populations of patients, which is unattractive to designers of clinical trials and enrolled patients. As a result there are no large-scale studies correlating clinical benefit, response or survival endpoints with PK parameters. Reported pharmacokinetic/pharmacodynamic relationships are summarised in Table 3.

Treatment	Tumour	n	variable	Finding
Carboplatin and FDR GEM (Wang et al., 2007)	NSCLC	21	% reduction WBC and GEM-CMax	R2= 0.4575
			% reduction plat and GEM CMax	R2= 0.5671
Paclitaxel GEM (Fogli et al., 2001)			% reduction plat and GEM CMax	
High dose GEM and autologous stem cells (Bengala, et al., 2005)	pancreas	23	Mononuclear CDA activity and expression	Low activity /expression associated with response
Gemcitabine and RT (Eisbruch et al., 2001)	Head and neck	29		Poor correlation between dose and tumour concentration

Table 3. Pharmacokinetic/Pharmacodynamic relationships

6. Population models of Gemcitabine PK

6.1 Population PK models

Pharmacokinetic models are a mathematical shorthand way of describing PK data. In the most common approach to estimation of pharmacokinetic parameters, data from an *individual* is used to fit to a model and calculate variables such as clearance. Individual estimates of clearance then describe the population. In the population pharmacokinetic approach the data from the whole *population* is used to develop the model parameters and calculate variables, like clearance. The population approach is more complicated and requires more computing and human resources but has several advantages including the handling of sparse data. It is well positioned to include other co-variates in the model development such as clinical parameters (age, BSA, measure of renal or hepatic function) as well as novel laboratory measures. In both cases the model "compartments" are mathematical constructs rather than physiological compartments. Population PK modelling does lend itself to conforming the model to physiological compartments, which may also be sampled, such as plasma or an intracellular compartment. For a review of population PK modelling of anticancer agents the readers is referred to the review by Zandvliet et al. (2008).

6.2 Population PK models for Gemcitabine

There are 3 reported population pharmacokinetic models for plasma Gemcitabine and dFdU and one model that includes GEM-TP. Each GEM model fits for a 2 compartment model with a presumed peripheral and central compartment. Jiang et al derived our model from 94 patients with various malignancy treated on PK studies. Sugiyama et al derived a model from a population of 250 Japanese patients included in previous PK studies. Zhang et al derived their model from the data from original phase 1 studies. The model parameters for all three models are summarised in Table 4.

Study	Compartments	CL L/min	VC (l)	VP	RV
STG	2	2.7 (31%)	15 (39%)		40
Sugiyama *		1.24	12.6	9.54	
Zhang	2	NR	NR	NR	
Pham	2	5.1	2.96	47.6	35

CL=clearance; VC= volume central compartment VP = volume peripheral compartment and RV = residual variation

*calculated assuming a BSA of 1.78, Cr=100mg/dL age 62.67 and median values for male and female

Table 4. Parameters from Gemcitabine Population Pk Models.

The models allow some significant observations. Significant covariates included in the final version of all models include BSA, age and sex. This confirms univariate observations from individual PK data. The inclusion of BSA is an important observation given the debate over whether clinical doses should be adjusted for BSA for anticancer drugs.

The models also allow evaluation of potential drug interactions with co-administered drugs. The Jiang model compared the impact of co-administered of oxaliplatin with a 35-45% decrease in the Vc for dFdU.

The patients in the Sugiyama paper were administered a variety of partner drugs and this allowed evaluation of potential interactions. They were able to demonstrate a 20% increase in the clearance of GEM following S1, a potentially clinically relevant observation. The speculative mechanism is an increase in hENT1 expression. This demonstrates the relevance of considering intracellular pharmacokinetics because an increase in clearance into the intracellular compartment (which is greater in tumours) has a different implication to an increase in clearance due to increased excretion. A summary of findings of each model for significant covariates is given in Table 5.

Author	n	Examined	Significant	Comments
Sugiyama	248	(BSA), weight, age and sex and co-administered drugs, cisplatin, carboplatin, fluorouracil, S-1, vinorelbine	Including BSA improved modelling of clearance S-1 significantly increased CL	Age and sex did not significantly affect the CL and V1 of Gemcitabine.
Zhang	353	age, sex,(BSA), type of cancer, renal function, rate, and duration of infusion, plasma CDA activity	BSA on Clpc, Vpc,and Vpp,gender on Clpc and Vpc,ageon Clpc, and duration of infusion on Vpp.	Note plasma CDA not significant
Tham	56	age, body size, (KPS), stage, creatinine clearance, sex, race, and Smoking history.	Weight and height	No improvement with allometric scaling; fat content can be ignored when using size variable. The influence of sex disappeared when considering lean body mass

Table 5. Co-variates examined versus included in GEM models

6.3 GEM Pop PK including pharmacogenetic variables

The Sugiyama model also investigated the influence of several pharmacogenetic variants in the Japanese population. They had previously reported a variant CDA 208G>A (associated with the*3 haplotype) with a minor allele frequency of 0.022 in the Japanese population as associated with increased toxicity in homozygous patients (Sugiyama, et al., 2009).

They investigated the role of this other polymorphisms in CDA, DCK and hent1 (SLC29A1) and evaluated included the following found common polymorphism in the base model CDA A79>C variant (allele frequency 0.208); –31delC (0.44); 2 polymorphisms in the hENT 1 gene–3797A>G (0.398) and –3268_–3249del AGGCTCGCGAGCGGAGGTGC (0.398). The

significance of the final model was driven by CDA*3 , although -31delc and CDA A79>C also contributed. The significance of the *2 haplotype in the absence of *3 (as is seen in Caucasians) is not clear from their model.

One interesting piece of data relating pharmacogenetic and PK comes from a very simple study where the deamination rate of red cell extracts was measured and correlated with genotype (Giovannetti et al., June xx 2008). Mean enzymatic activity was significantly lower in patients carrying the CDA Lys27Lys than in patients with the Lys27Gln or Gln27Gln protein. This highlights the potential of measuring activity in accessible targets (such as ex-vivo plasma) to model phenotype, and explores phenotype-genotype correlations.

6.4 Population PK including intracellular GEM-TP

Zhang et al were able to extend the population model to include estimation of GEM-TP. They used the existing plasma GEM model and added an extra compartment (the intracellular compartment). GEM-TP concentrations were explained by a simple Michaelis-Menten formula. No additional parameters were investigated and the Km was fixed at the median value and the infusion rate was fixed at 10mg/m2/min.

(Tham et al., 2008) also derived a population PK model with intracellular compartment by adding an extra compartment to the Gemcitabine plasma 2 compartment model. They concluded, in comparison, that the GEM-TP was equally as well explained by a linear model, and therefore saturable metabolism did not occur. Interpretation of their data is complicated because it appears that fixed dose rate and standard dose rate Gemcitabine data has been lumped together despite previous data that saturation only occurs with the standard dose rate regimen.

The disparity in interpretations also highlights the assumptions brought to bear in selecting pharmacokinetic models. Tham et al have selected the most parsimonious model and concluded that there was no evidence to support a saturable model; Zhang et al have fitted a Michaelis-Menten model based on previous data and found that it describes their data adequately. This reflects a basic debate about whether science is assumption free. Karl Popper has eloquently argued that all science is based on assumptions. Rejecting the saturable PK model on the basis of lack of improvement in the objective variable should not be taken to mean that saturable metabolism does not occur, just that in this data set the model did not improve performance.

6.5 Physiological modelling

A very sophisticated approach has been taken by (Battaglia & Parker, 2011) they have constructed an intracellular accumulation model based on existing plasma PK models. They have created a physiological based model of Gemcitabine intracellular accumulation with initial estimates based on cell line measurements. They have modelled each of the parameters as first order but the overall model produces saturable metabolism due to the interactions built in. The model includes a variable for the dose rate and includes such physiologic processes as self- potentiation and cell cycle specificity, and diffusion through the interstitial space into solid tumours.

This work demonstrates the potential of combining measurement of clinically relevant compartments with an understanding of the biology involved. One application of such

models is that it can be used to drive clinical trial design to determine the optimum conditions for maximising tumour exposure under a variety of circumstances. This can potentially lead to more efficient clinical trial design.

6.6 Modelling of response

The relationship of pharmacokinetics to response is much more difficult to assess as tumour reponses are heterogeneous and seen in a minority of patients treated with Gemcitabine. This then requires PK sampling in large populations' of patients, which is unattractive to designers of clinical trials and to enrolled patients. There is however one very interesting attempt to do this in the literature. Data from a randomised phase 2 study of fixed dose versus standard dose rate administration of Gemcitabine was used to provide PK data and measurements of individual tumours. The pharmacokinetic parameters were estimated from the previously derived 4 compartment population model. The tumour effect compartment was then modelled according to a delayed exposure to estimate tumour exposure. The inhibitory effect of drug exposure on tumour growth was then modelled (from the change in size of the tumour) by a Gompterzian tumour growth model. The impact of drug exposure on tumour inhibition was then tested by Emax, or sigmoid Emax, models with exposure estimated by either dose or estimated AUC. In the final model using Intra-cellular GEM-TP AUC did not improve the fit over using Gemcitabine dose. Interestingly the dose response variability of 146% was much greater than the interpatient variability in Gemcitabine clearance, suggesting that a target concentration approach would not be helpful in dose individualisation.

This work represents a "heroic" attempt to take the PK/PD modelling to its logical extreme, however the interpretation of the observation that intra-cellular GEM-TP did not improve the –prediction of response should be coloured by a realisation of the limitation of the model. Firstly the PK model, the tumour growth model and the final PK/PD model are all based on data from a relatively small number of patients. Secondly the measurements that they are based upon e.g. GEM-TP and tumour size –have relatively large errors. Thirdly the three inter-related models are all based on the assumptions outlined in the paper. Many of these are unlikely to be true, such as the independence of Gemcitabine and Carboplatin. Finally the significance of the observation that a particular co-variate does not improve the fit of the model does depend on the overall fit of the model. Obviously a very poor model will not be sensitive to variations in the inputs chosen; it will perform equally well – or equally poorly. In this regard the overall model has very wide confidence interval the median tumour size at 12 weeks is approximately 4cm with a 95% confidence interval of 2.5-12cm, clinically very significant differences. The relevance of the Gomptzerian model is also in question as it is now very clear that a major part of the benefit of cytotoxic therapy relates to the achievement of prolonged stable disease and tumour dormancy rather than tumour response. Nevertheless this work is a very exciting demonstration of the possibilities of incorporating tumour response into well understood pharmacokinetic models.

6.7 Discussion

These data demonstrate some of the possible applications of a population PK approach. The limitations of each model also demonstrate the potential of using models, which are one step closer to the source.

The Sugiyama model is a very exciting step towards the incorporation of pharmacogenetic data to inform model development. Any pharmacogenetic model will be constrained by the population it is developed in hence the dominant effect of the *3 mutation, which is extremely infrequent in the Caucasian population, may obscure the effects of other polymorphisms in other populations.

The major step towards developing a physiologically based model would be to apply the polymorphisms to the model in a physiologically informed way. CDA is going to be the main determinant of plasma clearance, so its incorporation into the elimination from the central or peripheral compartment is most relevant. However DCK and hENT1 are determinants of uptake into the intracellular compartment. The greatest potential then would be to model them as variants of the transfer into the intracellular compartment.

A further step towards more informative models would be to use preclinical data to inform initial model parameters for variables such as the relationship between GEM-TP accumulation and drug concentration.

7. Discussion and future directions

Gemcitabine provides a model for the study of intracellular pharmacokinetics as a way of moving one step closer to the target. The improved correlation of GEM-TP pharmacokinetics with pharmacodynamic endpoints such as dose reduction or leukocyte nadir is proof of principle that this provides important additional information. Given the accessible nature of this compartment it is difficult to see why correlative studies should continue to pursue plasma pharmacokinetics as an endpoint.

However the data presented also demonstrates the limitations of intracellular-pharmacokinetics. The tissue sampled in this literature is leucocytes, which are presumed to be a surrogate marker of normal tissue and or tumour uptake. The correlation with other normal tissues is the less problematical assumption, as the genotype is the same. It is worth considering in what ways leucocytes are different from other target tissues such as bone marrow progenitor cells.

Firstly the regulation and expression of genes is tissue specific with upregulation of DCK and CDA in tumour tissue (Spasokoukotskaja et al., 1995; van der Wilt et al., 2003). Lack of correlation between genotype and phenotype is an issue with highly regulated genes such as CYP450, which are induced and inhibited by drugs and environmental exposures (Shiran et al., 2003). Secondly issues around tissue penetration into compartments such as the CSF, brain, etc are highly relevant for tumour metastases. Finally the tumour microenvironment is one that provides multiple barriers to drug access such as tumour hypoperfusion, increased interstitial pressure (Willett et al., 2004) and stromal reactions around tumours(Minchinton & Tannock, 2006). There is some evidence that these factors are critical in determining intratumoral and intertumoural heterogeneity in drug exposure (Zamboni et al., 2002; C. A. Presant et al., 1994).

Even given these limitations there are still a number of significant opportunities associated with the ability to measure pharmacokinetics in the intracellular compartment. The first is the ability to assess the clinical significance of availability in genes of known importance. As summarised above there are a great number of potential sources of

variability in intracellular Gemcitabine uptake. However the source of human variability in uptake is an empirical question. Early pharmacogenetic evidence already suggests that this may be population specific (Fukunaga et al., 2004). Measurement of intracellular pharmacokinetics allows questions to be asked regarding correlation between variation in genes such as CDA and intracellular uptake. The time taken to perform studies with surrogate makers such as intracellular uptake is much shorter than those requiring clinical endpoints such as survival. This allows prioritisation of potential markers for prospective validation.

A second possibility for the utility of intracellular pharmacokinetics is in the development of strategies for therapeutic dose monitoring. Moving closer to the target increases the possibility of developing an effective TDM strategy. However data from pharmacodynamic modelling suggest the greater part of interpatient variability comes from pharmacodynamic variables suggesting limited value from pharmacokinetic monitoring (Tham et al., 2008). Related to this is a third possibility of pharmacogenetically derived dosing where known polymorphisms in genes of interest affects the dose or scheduling of the drug administered (Ciccolini et al, 2011).

A significant challenge relates to application of pharmacogenetic strategies where drugs are given in combinations. This requires a clear understanding of the effects of the drugs on each other, for example the effect of DNA damaging agents on Gemcitabine accumulation (Metharom et al, 2010) and the conditions under which drugs act synergistically (Luk et al, 2011).

Optimising the dosing of Gemcitabine according to a rational understanding of an individual patients threshold for saturation of GEM-TP is a rational strategy for reducing toxicity and improving efficacy.

A conventional strategy for optimising dose is to build upon a population pharmacokinetic model where clinical or laboratory variables of interest are correlated with clinical outcomes. The next step with such a model would be to incorporate pharmacogenetic variables of interest such as measurement of CDA genotype of phenotype. Determining the coefficients by which the impact of such variables can be adjusted will require very large clinical studies. Intracellular PK measurements offer the possibility of providing initial estimates of such variables from smaller correlative studies both in vivo and in-vitro. The advantage over other scenarios such as liver oxidation or glucuronidation is that the genes are measurable and the pharmacokinetic intermediate is assessable. This is an important area of inquiry to lead us to a goal of a pharmacogenetically guided individualised dosing strategy.

These strategies are of course complimentary to those aimed at measuring drug distribution directly in tissues of interest by the use of tracer radio-pharmaceuticals, microdialysis, NMR or PET. The common theme is that facilitating the contribution of pharmacokinetics to optimising drug administration in oncology requires us to move closer and closer to the target of interest. A variety of techniques to measure intracellular uptake as well as tissue penetration and access to the tumour microenvironment are likely to be required to minimise the effect of drug variability on the response to anticancer treatments. Combining mesurement of more relevant compartments with physiologic modelling and incorporation of pharmacogenetic variation, raises a real possibility of predicting drug response in a way that realises the hopes of individualised medicine.

8. Appendix

Background information on Single Nucleotide Polymorphisms

Finding information
The NCI maintains a database of polymorphism (dbSNP) [1]

Nomenclature
Polymorphisms can be referred to by their unique identifier (rs number)
but are preferably described at the DNA level[2] for example
Rs2072671 describes the polymorphism CDA c.79A>C

- which occurs in the CDA gene:
- where the c stands for coding sequence;
- 79 is the first nucleotide affected;
- and the nucleotide A is substitute for C.

This results in a protein change described as p.K27Q where the p stands for protein and the K and Q are exchanged at the 27th amino acid position. Nomenclature for haplotypes becomes more complicated. Because of the sometime complicated nomenclature for complex polymorphisms or haplotypes they are usually given nicknames e.g. *1, *2 etc.

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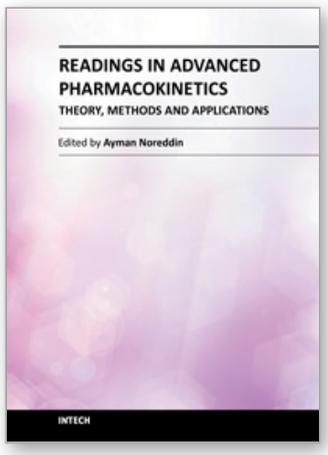

Readings in Advanced Pharmacokinetics - Theory, Methods and Applications

Edited by Dr. Ayman Noreddin

ISBN 978-953-51-0533-6
Hard cover, 378 pages
Publisher InTech
Published online 20, April, 2012
Published in print edition April, 2012

This book, "Readings in Advanced Pharmacokinetics - Theory, Methods and Applications", covers up to date information and practical topics related to the study of drug pharmacokinetics in humans and in animals. The book is designed to offer scientists, clinicians and researchers a choice to logically build their knowledge in pharmacokinetics from basic concepts to advanced applications. This book is organized into two sections. The first section discusses advanced theories that include a wide range of topics; from bioequivalence studies, pharmacogenomics in relation to pharmacokinetics, computer based simulation concepts to drug interactions of herbal medicines and veterinary pharmacokinetics. The second section advances theory to practice offering several examples of methods and applications in advanced pharmacokinetics.

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