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Recent developments in the clinical pharmacology of classical cytotoxic chemotherapy

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Advances in analytical methods, imaging techniques and an increased understanding of the influence of pharmacogenetic factors have added to our knowledge of the pharmacology of many chemotherapeutic agents. Extending the use of these approaches to pharmacodynamic end-points, together with the application of population-based modelling techniques, offers the potential to develop truly individualized therapy in the future.

The concept that major improvements in cancer treatment could come about simply by increasing our knowledge of the systemic pharmacology of cytotoxic drugs has not been borne out by many of the studies performed in the last 20 years. Data on plasma concentrations of drug and metabolites have allowed the successful development of a number of novel agents and has been useful in guiding dosage regimens and the design of novel prodrugs. However, the goal of using clinical pharmacology to individualize therapy in order to optimize the treatment of each tumour in every patient has not been achieved. Clinical pharmacology studies continue to generate more detailed data on classical cytotoxic drugs. However, to date only for carboplatin [1, 2] and possibly for methotrexate [3] and etoposide [4] is there any rationale for individualized dosing based solely on a knowledge of systemic pharmacology. In this review, I will focus on the methodological and conceptual developments that have augmented our understanding of 'classical cytotoxic' drugs, beyond simple measurements of total drug or metabolites in plasma. These developments include new technologies, integration of genetic and other biological determinants of pharmacology and novel statistical and data analysis approaches.

For the purposes of this discussion, classical cytotoxic chemotherapy is defined as treatment involving agents that have a nonspecific effect on cell division or cause cell death by means of damaging DNA, directly or indirectly. Many of these drugs were applied clinically before any detailed knowledge of their mechanism of action was available. To this extent, classical agents are distinct from more recently developed, targeted approaches, where drugs are designed specifically to interact with a defined oncogenic target [5, 6]. Although lessons learnt from the pharmacology of 'classical' cytotoxic drugs may have some relevance for more specific agents, these do not relate to any specific pharmacological properties.

Recent advances in clinical pharmacology can be broadly divided up into four main areas. These are:

1 Systemic pharmacology, based on measurements of drug or metabolites in blood. Advances in this area include improvements in analytical methodology and advances in pharmacokinetic modelling of data.
2 Pharmacodynamic end-points. This is a broad area taking in measurements of drug action in blood and possibly in the tumour tissue itself. Other advances in this area include development of novel imaging techniques for drug, metabolites and surrogate markers.
3 Pharmacogenetic and gene regulatory determinants of response and toxicity have been investigated for a number of drugs. Many more polymorphisms in enzymes and transporter proteins relevant to cytotoxic drugs have now been identified and there have been developments in the techniques applied to identify such genetic variants and to assign genotypes. The influence of epigenetics on response of tumours to drug treatment has also been increasingly recognized.
4 The mathematical techniques applied to pharmacokinetic modelling have recently been extended to incorporate models of pharmacodynamic response. Future developments in this area are likely to include elements of the other three areas mentioned above.

I will deal with each of these areas in turn, but it is important to recognize the extensive areas of overlap between them and the potential for integration of each of these developments.

Systemic pharmacology, analytical methodology and pharmacokinetic modelling

The last 15 years has seen significant developments in the analytical methods applied in cancer pharmacology [7, 8]. Most notable in these developments has been the advent of affordable, bench-top mass spectrometry as a method of detection, combined with liquid chromatography (LC-MS) [9]. Such instrumentation is now available to the nonspecialist with minimal training. The technique of LC-MS makes possible the determination of nanomolar or picomolar concentrations of drugs in body fluids (e.g. vincristine [10], actinomycin-D [11], cyclophosphamide [12, 13]). This increase in sensitivity permits the detection of species present at low concentrations, such as the active 4-hydroxy metabolites of cyclophosphamide [12] or 5-fluorouracil (5-FU) formed from capecitabine [14]. There is also the potential to use microlitre volumes of plasma in assay methods, which is of particular importance in studies in children with cancer (e.g. for etoposide [15]). Finally, the greater sensitivity and specificity of LC-MS over other LC detection methods means that drug and metabolites can be analysed in cells or tissues other than whole plasma, for example unbound etoposide [15] or cisplatin [16] in plasma water, methotrexate in cerebrospinal fluid [17] or fludarabine in leukaemic cells [18].

A related development is the combination of time-of-flight (TOF) mass spectrometers, which can be combined with a variety of sample separation and presentation methods to provide high-resolution mass spectra from biological samples. Such TOF instruments have applications in areas including metabolomic [19] and proteomic profiling [20]. Profiling of proteins and other biochemicals may be useful in characterizing tumours [21], detecting tumour material in peripheral blood or characterizing the response of a patient to a particular drug, by comparing profiles in tumour or blood before and after treatment [22].

Both the metabolism and mechanisms of action of anticancer drugs are often complex. Using pharmacokinetic modelling to gain insights into the action of these drugs is complicated by the low concentrations and unstable nature of important metabolites and by the fact that the relevant biological site is often removed from the most convenient site to sample (i.e. plasma). Many drugs are processed intracellularly and, although accessible cell populations such as peripheral blood lymphocytes (PBL) or erythrocytes [23] may serve as a surrogate, metabolism in tumour cells may differ from that in normal tissues. Indeed, many drugs rely on such differences to achieve selectivity towards tumour cells [24]. Population pharmacokinetic techniques have been employed to generate sophisticated models which provide information on the systemic and tumour pharmacology of many drugs used in cancer [25–28]. Incorporation of pharmacogenetic covariates (see below) and other biochemical information has increased

the predictive power of these models in the individualization of cancer treatment [29].

A number of models have been developed in an attempt to predict the concentrations of active metabolites in tumour cells [26, 30]. These models have been developed with knowledge from *in vitro* experiments and animal tumour models [31, 32]. However, extrapolating to human tumours inevitably involves a number of assumptions [33]. Population approaches have also been successfully applied to the modelling of haematological toxicities (see below). The incorporation of data from novel sophisticated imaging methods (see below) will, it is to be hoped, improve the utility of these models.

Pharmacodynamic end-points

In cancer chemotherapy, pharmacodynamic end-points have always provided challenges and hurdles in understanding the pharmacology of cytotoxic drugs. The end-point that matters is, ultimately, the survival of the patient. Intermediate end-points, such as shrinkage or disappearance of the tumour, may be predictive of long-term survival. Classification of complete or partial responses is dependent on appropriate imaging or other tumour detection methods. In routine clinical practice, the assignment of a pharmacodynamic end-point to the action of a single drug is often complicated by the use of multiple agents in a single regimen.

Developments in detection methods for tumours, such as the ability of polymerase chain reaction to detect the Bcr-Abl gene in chronic myelogenous leukaemia (CML) [34, 35], have redefined the most relevant end-point from complete haematological remission to a so-called molecular remission. That development, in turn, has become meaningful only with the development of imatinib as a novel effective treatment for CML [6]. The development of imatinib, which inhibits the tyrosine kinase activity of the Bcr-Abl protein, is an example of a novel cancer treatment directed towards a known biochemical target selective to tumour cells. As the prototype for other such targeted therapies, imatinib is in contrast to conventional cytotoxic drugs that, at best, exploit the higher proliferation rates of tumour cells over normal tissues.

Investigations of pharmacodynamic end-point with conventional agents have relied on detection and quantification of drug–target interactions. For many of these drugs, the target is DNA and pharmacodynamic methods have been developed to measure DNA adducts, DNA damage or incorporation of nucleoside analogues into DNA [36]. Thus, a number of techniques have been developed to measure platinum DNA adducts, in the case of cisplatin, carboplatin and other analogues [37–39]. These techniques have been mainly applied to DNA from surrogate tissues such as PBL. The covalent binding of alkylating agents to DNA results in DNA cross-links or strand breaks. These lesions in DNA may be detected indirectly using the COMET assay [40]. So far, most studies have found little or no relationship between the degree of adduct formation and either pharmacokinetic end-points such as the area under the plasma concentration time curve (AUC), or clinical outcome (toxicity or antitumour effect). As with DNA reactive drugs, the incorporation of nucleotide analogues into DNA has been quantified in surrogate tissues such as PBL or erythrocytes. Such measurements has been possible for 6-mercaptopurine [23], gemcitabine [41, 42], fludarabine [18] and cytarabine [43].

Measurements in PBL have some worth in demonstrating that the drug has been appropriately activated and is able to interact with the target. However, there is no information regarding the effects of the drug in the tissue of interest, i.e. the tumour. Advances in imaging techniques have permitted some modest insights into the pharmacology of antitumour agents in tumours in patients [44]. The main advances in imaging have been the widening availability of positron emission tomography (PET). The diagnostic use of PET to grade and determine the response of solid tumours is now established practice in many countries [45, 46]. Research applications of PET are still limited by the short half-life of positron-emitting isotopes and the scarcity of cyclotron facilities. Nevertheless, a number of interesting studies have been performed. Labelling of the methylating group of temozolomide with ^{11}C (half-life 20 min) has shown that temozolomide methylates DNA in brain tumours and also shows the time course of repair of this lesion [47]. Thymidine labelled with ^{11}C can be used to demonstrate that tumours increase thymidine salvage when thymidylate synthase is inhibited, suggesting a number of approaches for augmenting the activity of these drugs [48]. Since fluorine has one of the more stable positron-emitting isotopes, labelling a compound with ^{18}F (half-life 110 min), either at an existing fluorine or as a fluorine analogue, has been used in a number of studies. The major diagnostic use of PET uses ^{18}fluoro-deoxyglucose [46]. Drugs such as 5-fluorouracil have been studied using PET [49, 50]. The complex data derived from these experiments require intensive mathematical models to distinguish vascular background and the contribution of metabolites from the signal for parent drug [51]. This highlights one of the current drawbacks with PET, that parent drug and any metabolite retaining the positron-emitting label will be detected indiscriminately.

Fluorine chemistry provides the opportunity for another potentially useful imaging technique. Magnetic resonance spectroscopy (MRS) imaging provides structural identity information, as well as some quantitative data on compounds which have a fluorine group [52]. This approach has been applied to 5-fluorouracil [53, 54], but is limited to tumours near to the body surface, due to the limited range of the MRS signal. Phosphorus is another possible nucleus which may be suitable for *in vivo* MRS imaging and has been applied to the study of ifosfamide and cyclophosphamide metabolism on urine samples [55] and in tumours. So far, results with MRS have been rather limited, due to the narrow range of compounds for which the technique is appropriate and limits on sensitivity and range of the available scanners.

Genetics and regulation of drug-metabolizing enzymes, transporter proteins and cytotoxic response genes

Advances in this broad category depend on an increased understanding of the role of drug metabolism in the activation and inactivation of cytotoxic drugs, the role of transport proteins in determining hepatic uptake and biliary and renal excretion and the role of DNA repair and other proteins in modulating the response to cytotoxic drug action.

It has long been known that CYP enzymes are important in the activation and inactivation of drugs such as cyclophosphamide, docetaxel and etoposide. However, attempts to individualize therapy based on phenotypic enzyme activity, usually based on the elimination of a marker compound, have met with only limited success [56]. The role of genotype in a number of well-characterized polymorphisms in CYP2B6, CYP2C8 and CYP3A4 and 3A5 is now being investigated in the context of the pharmacology and clinical effect of cyclophosphamide [57, 58] and paclitaxel [29], with some intriguing preliminary results. Similarly, a critical role for UGT enzymes in the inactivation of camptothecins has suggested that dose individualization of irinotecan could be achieved by determining the number of TATA repeats in the regulatory region of the UGT1A1 gene [59], or by genotyping for polymorphisms in UGT1A7 or 1A9 [60].

These pharmacogenetic investigations have built on the examples of polymorphisms in dihydropyrimidine dehydrogenase (DPD) [61] and in thiopurine methyltransferase (TPMT) [62], influencing the pharmacology of 5-fluorouracil and 6-mercaptopurine, respectively. The inactivating genetic variants in DPD and TPMT are relatively rare and attention is now focusing on more frequent variants, with influences on pharmacology that are likely to be more subtle than complete ablation of a metabolic pathway. The role of single nucleotide polymorphisms (SNPs) in other genes which are involved in the systemic and cellular pharmacology of drugs such as nucleotide analogues [63], antifolates [64], bioreductive agents [65] and substrates for various drug transports [66, 67] is now under investigation. Many of the SNPs are in regulatory regions of the genes involved and the regulation of the corresponding enzymes and transporter proteins is an area of increasing interest and activity.

Regulation of other genes whose protein products influence response to treatment is another area where there have been some exciting recent advances. The repair of DNA methylation by the enzyme methylguanine methyltransferase (MGMT) has long been associated with resistance to temozolomide. Tumours with high expression of MGMT are relatively resistant to temozolomide in cell lines, xenografts and in clinical studies. Several recent studies have reported that silencing of the MGMT gene by promoter methylation in tumours is associated with a more favourable response to temozolomide treatment [68, 69]. Resistance to temozolomide may also result from a deficiency in one of the mismatch repair proteins, which have been shown to have an important role in the recognition and processing of alkylated DNA lesions [70, 71]. Loss of mismatch repair function is linked to failure to undergo apoptosis and silencing of MLH1 is due to promoter methylation [71]. Thus, reversal of MLH1 promoter methylation may improve the response to temozolomide and to other drugs, such as cisplatin or carboplatin, which require an intact mismatch repair pathway to cause a cytotoxic effect. Drugs such as decitabine, which inactivate DNA methyltransferase enzymes, can restore the sensitivity of tumour cells previously resistant to platinum drugs [72]. Clinical studies of decitabine in combination as a demethylating agent are ongoing [73]. Obviously, there is a contradiction here, in that methylation of MGMT is a good thing in blocking a DNA repair pathway, whereas methylation of MLH1 is a bad thing as that pathway is required for an antitumour effect. The exact role of gene methylation and the role of other epigenetic factors such as histone acetylation require further research before they can be exploited clinically to full effect.

Integrated modelling of pharmacokinetic, pharmacodynamic, pharmacogenetic and imaging data

Pharmacodynamic modelling in cancer pharmacology is handicapped by the dissociation, both temporal and physical, between drug presence in a measurable com-

partment (often plasma) and the pharmacological effect. As noted above, major toxicities such as myelosuppression or diarrhoea occur hours or days after detectable drug has been eliminated from plasma. Likewise, any measurable antitumour effect will not be seen for days or weeks after drug administration. To identify any relationship between plasma concentrations of drug and either toxicity or antitumour effect, investigators have resorted to summary measures of drug exposure (e.g. AUC or time above threshold concentration). For instance, the pharmacological effects of carboplatin have been closely correlated with AUC for ultrafiltrable platinum [2]. Paclitaxel haematological toxicity and antitumour effect in ovarian cancer have been associated with the time for which plasma concentrations exceed 0.05 μM [74].

More recently, physiological models of haematopoiesis or tumour growth have been combined with pharmacokinetic data to generate holistic models of drug action, which may be predictive of pharmacological effects for a number of drugs [75, 76]. Whether these models can be extended to incorporate the combined effects of a number of agents used in combination is not yet clear. Likewise, the incorporation of genotype as a covariate in population models has been reported for irinotecan [77] and paclitaxel [29]. These models may be useful in identifying genotype influence on the phenotype of drug metabolism. A further step would be to combine all of the above information, perhaps together with data from PET or magnetic resonance tumour imaging.

While the logical extension of combining all of the above modelling approaches to achieve a single integrated model of drug action may appear attractive, it should be recognized that the uncertainty involved in each of the components may be so large as to make such a model meaningless. Intersubject and interoccasion variability in the magnitude and nature of the influences on drug effect mean that any model developed in a particular population may lack wider application outside that patient group. Nevertheless, important information concerning the mechanism of action of a drug and the influence of important factors affecting drug action may be gleaned by such an approach. With the advent of microarray-based approaches, information on genes and proteins that influence drug action will be increasingly available, as will information on those genes and proteins which are in turn affected by the drug itself. Managing all of these data represents an increase in the order of magnitude of data that could be explained by a particular pharmacological model. Such a task would require the development of ever more sophisticated statistical and mathematical techniques, alongside a corresponding increase in computing power.

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