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Review

Pharmacokinetics in cancer chemotherapy

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1. Introduction

Pharmacokinetics is the study of how the body causes changes in drugs, and includes analysis of absorption, distribution, metabolism and excretion.

It is essential to understand pharmacokinetics to establish the route of administration, the dose and schedule of treatment of a drug, the interactions when more than one drug is given and the relations between drug concentrations and biochemical or functional effects (pharmacodynamics).

The presence of a tumour alters many parameters in the body so the pharmacokinetics of antitumoural drugs cannot be extrapolated from normal people to patients with tumours. Furthermore since the tumour is the target of an antitumoural drug it is important to know drug concentrations in that particular tissue. In addition, since the tumour grows and its sensitivity to drugs changes with time it is desirable to know how the pharmacokinetics change in relation to the formation, growth and dissemination of metastases.

Unfortunately pharmacokinetic studies are often done mainly for regulatory purposes rather than as part of the studies that should accompany all the various steps of antitumoural drug development. Often this lack of data explains the failure of promising drugs which prove ineffective or toxic, but it can also lead to false-negative results.

This mini-review is not intended as an exhaustive analysis of all the studies on the pharmacokinetics of antitumour drugs. It discusses some special issues, such as the host and tumour factors that determine drug concentrations in tumours. Particular attention is paid to questions such as primary versus metastatic tissues; penetration of drugs into the various layers of a tumour; importance of metabolites in the efficacy of drugs; cellular and intracellular transport of antitumoural drugs, also in relation to resistance.

2. Anticancer drug concentrations in tumours

Technological developments have made it possible to measure with high sensitivity and precision drug concentrations in blood and tissues not only in biological samples[1] but also *in vivo* through the use of PET, MRI[2] and detectors of luminescence and fluorescence.[3] Usually drugs are determined in blood because it is easily accessible, and more rarely in tissues. Blood drug concentrations need to be known for adjusting doses in order to reach the predicted therapeutic or safe concentrations.[4–7] However, studies in animals have clearly demonstrated that blood concentrations of antitumoural agents are not always predictive of their concentrations in tissues,[8–10] particularly in tumours. In some cases comparable blood levels lead to completely different tumour concentrations.

As a general rule, the concentrations of antitumoural agents in normal tissues are higher than in the primary

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BOX 1. HOST FACTORS INFLUENCING DRUG CONCENTRATIONS IN TUMOURS

- – AGE, SEX, BODY WEIGHT, RACE, CO-MORBIDITY
- – GENETIC DIFFERENCES IN CYTOCHROME P450 AND DRUG EFFLUX AND UPTAKE TRANSPORTERS
- – INTERACTIONS WITH HERBAL REMEDIES, FOOD AND DRUGS

ABSORPTION
- – NAUSEA, VOMITING
- – GASTRIC ACID SECRETION
- – DISSOLUTION OF TABLETS

DISTRIBUTION
- – ASCITES OR PLEURAL EFFUSION
- – PLASMA ALBUMINS AND ∝-ACID-GLYCOPROTEIN
- – AMOUNT OF BODY FAT
- – BLOOD BRAIN BARRIER

METABOLISM
- – LIVER DYSFUNCTION
- – ALTERED HEPATIC BLOOD FLOW
- – REDUCED LIVER MASS
- – INFLAMMATION

EXCRETION
- – ALTERED BILIARY FLOW
- – RENAL INSUFFICIENCY
- – URINARY PH

tumour.[6,11–14] Like blood levels,[15] the concentrations in tumours are highly variable[16,17] and this may also explain the variability in responsiveness to antitumoural drugs. In some cases, however, similar tumour concentrations inhibit tumour growth to different extents.[18]

These examples clearly indicate how important it is to measure anticancer drugs in tumours so as to interpret the therapeutic results correctly. It is therefore essential to define which factors govern the distribution of drugs or their metabolites in tumours (see Box 1).

3. Host factors affecting tumour concentrations of antitumoural drugs

Several anticancer drugs are administered intravenously to avoid the problem of intestinal absorption which may be highly variable due to factors such as the rate of tablet dissolution, diet, vomiting, intestinal mobility and flora.[15] In some cases, the intestine is a real barrier to drug absorption because of the presence of cytochrome P450 (particularly CYP3A3) in the enterocyte, which causes the metabolism and inactivation of drugs before they can be absorbed.[19] Another barrier is the transmembrane drug efflux pump like PgP,[20] which prevents the oral absorption of drugs such as paclitaxel. Blockade of PgP results in increased absorption of the antitumour drugs.[21] A new antitumoural agent, IDN 5109, is the first taxane derivative with good oral availability because it interacts poorly with PgP.[22] An analysis of the oral absorption of drugs that are substrates of PgP has been recently published.[23]

Impairment of liver or kidney functions may require reductions of standard doses due to the reduced metabolism and excretion, which can change activity and/or toxicity. Biliary excretion is an important route of elimination for several drugs. For instance, 4-glutathionylcyclophosphamide is transported through the canalicular membrane of hepatocytes by a multidrug resistance protein known as ABCC.[24,25] Rats deficient in this protein do not eliminate the metabolite, leading to accumulation of hydroxylated species toxic for the liver.[26]

Protein binding dictates the levels of free drug which are important for tissue distribution. Pharmacokinetic parameters may be influenced by such parameters as age, sex, ethnicity, body weight, concomitant diseases.[15] Other agents such as rifamycin can induce intestinal CYP3A, reducing the absorption of antitumour drugs.[19] These aspects are extensively reviewed in several texts such as.[15,27,28]

4. Drug metabolism

How metabolite formation influences the overall concentration and efficacy of a drug in tumours has not been widely investigated. Drugs like cyclophosphamide, used in many, if not most, chemotherapeutic combinations, have no direct effect on cancer cells *in vitro* and requires metabolic activation.[29] The antitumour effect of cyclophosphamide *in vivo*[30] requires the formation of at least two active metabolites, 4-hydroxycyclophosphamide and phosphoramide mustard. These are generated by the cytochrome P450-dependent enzymes CYP2B10, CYP2C29 and CYP3A13.[31,32]

Similarly, irinotecan requires the formation of an active metabolite, 7-ethyl-10-[4-N (5 amino pentanoic acid) 1-piperidino] carbonyl oxycamptothecin (ISN-38), via the enzyme CYP3A4.[33] Brostallicin, a synthetic ∝-bromoacrylic, second generation DNA minor groove binder[34–36] is interesting because its antitumoural activity is greatly enhanced by binding to glutathione. This is the consequence of glutathione-S-transferase (GST) activity (GST-M1-I and GST-P1-I). These results were confirmed *in vivo* with tumours expressing different levels of GST[37] and indicate how metabolic modifications of antitumour drugs may affect their levels of efficacy.

Other antitumoural drugs that require the formation of active metabolites to be active are listed in Box 2. In several

BOX 2. SOME ANTICANCER DRUGS ACTING THROUGH ACTIVE METABOLITES

CYCLOPHOSPHAMIDE	4-HYDROXYCYCLOPHOSPHAMIDE AND PHOSPHORAMIDE MUSTARD[30]
IRINOTECAN	OXYCAMPTOTHECIN (SN-38)[33]
BROSTALLICIN	BINDING WITH GLUTATHIONE[37]
TEMOZOLAMIDE	MTIC[5]
IFOSFAMIDE	4-HYDROXYISOFOSFAMIDE, ISOPHOSPHORAMIDE MUSTARD[189]
TAMOXIFEN	ENDOXIFEN[39]
CYTARABINE (ARA-C)	ARA-CTP[117,118]
CLADRIBINE	CLADRIBINE –5′– TRIPHOSPHATE[120]
GEMCITABINE (dFdC)	dFd-CTP[190]
CAPECITABINE	5′-DEOXY-5-FLUOROURIDINE-TRIPHOSPHATE[191]

cases, however, the metabolism leads to the formation of inactive metabolites. Thus the recognition of the metabolic steps involved in the inactivation of anticancer agents may be important to find inhibitors to increase their availability in neoplastic tissues.

5. Drug interactions

An important factor that may alter the availability of anticancer agents in tumours is concomitant or sequential treatment with other drugs. This is frequent given the widespread use of polychemotherapy protocols for cancer treatment.

Most of the interactions occur at the metabolic level. Ifosfamide, for example, markedly reduces the formation of the irinotecan active metabolite SN-38[33] in children. In breast cancer patients, doxorubicin markedly increases the plasma concentration of docetaxel. The effect is independent of the sequence of administration; doxorubicin levels are not affected.[38] Paclitaxel and docetaxel raise the concentration of epirubicin in mouse heart.[38]

Very little is known, however, about the interactions between the new target-drugs, e.g. monoclonal antibodies, and cytotoxic agents, which may lead to changes in drug distribution within the tumour. Interactions may also occur between anticancer drugs and drugs for concomitant diseases or symptoms. For instance, antidepressants, like the selective serotonin reuptake inhibitors (SSRI), markedly reduce the formation of endoxifen, the active metabolite of tamoxifen, probably through inhibition of CYP2D6.[39] Extracts of *Hypericum perforatum*, used to treat depression, reduce the formation of the active metabolite of irinotecan.[40] Grapefruit juice affects the absorption of several drugs because of inhibition of CYP3A.[19,41] Other agents, such as rifamycin, can induce intestinal CYP3A, with a consequent decrease in the absorption of antitumour drugs.[19]

6. Genetic polymorphism

While awaiting a genomic-based approach which may allow personalised anticancer therapy, information on the polymorphism of genes involved in drug metabolism is already available. Many variants of CYP2D6 identify poor, intermediate, extensive and ultrarapid metabolisers.[19] For instance, variant alleles of the genes encoding cytochrome P450 enzymes dictate the rate of metabolism of anticancer agents.[42–44] Variants of UGT1A1 which lead to decreased glucuronidation correlate with irinotecan toxicity due to accumulation of the active metabolite SN-38. Patients homozygous for the TA7 allele are at high risk of severe neutropenia.[45]

Thiopurine-S-methyltransferase (TPMT) is a cytosolic drug metabolising enzyme that catalyses the S-methylation of 6-mercaptopurine.[46,47] The variant allele TPMT*3A codes for an inactive enzyme, which exposes homozygous patients treated with thiopurine[48] to life-threatening toxicity.[49,50]

7. Drug metabolism and inflammation

Inflammatory responses are common in almost all types of cancer.[51–53] Components released from the cells involved in inflammation, namely cytokines such as IL1, TNF, interferon[54,55] and IL-6,[56] are probably responsible for the decrease of liver cytochrome P450[57] in tumour-bearing animals. A number of experiments have established that the metabolism of several drugs is reduced in tumour-bearing animals.[57–62] Sometimes this impairment is masked by concomitant administration of drugs such as rifampicin, which induces cytochrome P450.[63] An inverse correlation between inflammation and metabolism has been demonstrated in cancer patients. Decreases in CYP3A4 were associated with reduced clearance of docetaxel or vinorelbine and the effect was inversely correlated to the levels of C-reactive protein or α-acid glycoprotein,[53] two markers of inflammation in tumour patients. High levels of α-acid glycoprotein in blood may sequester antitumour drugs and reduce their availability, as shown for imatinib.[64]

8. Tumour factors affecting tumour concentrations of antitumoural drugs

Tumours themselves may influence the amount of drugs available for the neoplastic tissue. The type of tumour is important. The same dose of fotemustine, but not BCNU, resulted in different tumour AUCs in two lines of the Walker carcinosarcoma.[65] Doxorubicin is not measurable in sarcoma 180 at doses that give a measurable concentration in other tumours.[66]

The presence of ascites[67] may change plasma levels of drugs such as altretamine; similarly a pleural effusion may drain considerable amounts of etoposide or teniposide, making less available for the primary tumour[68] (Box 3).

9. Vascularity

The vascularity of tumours is certainly a determinant of the local concentrations of anticancer drugs. In constrast the lymphatic system is of minor importance given the scant presence of lymphatic vessels in the neoplastic mass.[69,70]

BOX 3. TUMOUR FACTORS INFLUENCING DRUG CONCENTRATIONS IN TUMOURS

INTRATUMOURAL BLOOD FLOW
- NUMBER OF VESSELS, ARCHITECTURE, PERMEABILITY
- FENESTRATION, INTRAVASCULAR THROMBI

TUMOUR CELL DENSITY
- THREE-DIMENSIONAL MEASUREMENTS
- LOCALISATION OF METASTASES
- VEGETATING VERSUS NECROTIC TISSUE

INTRATUMOURAL DRUG DISTRIBUTION
- DEFECTIVE LYMPHATIC DRAINAGE
- EXTRACELLULAR MATRIX COMPOSITION
- COLLAGEN CONTENT
- INTERSTITIAL PRESSURE

TUMOUR METABOLISM
- CYTOCHROME P450
- LEVELS OF ABC CASSETTE TRANSPORTERS (MDR, MRP)
- LEVELS OF NUCLEOSIDE TRANSPORTERS (ENT, CNT)

The tumour vascular network is irregular in its anatomical architecture; it contains blood thrombi[71,72] and has a low blood flow.[73,74] The vascular deficiency is accentuated as the tumour expands, causing a decrease in the vascular bed[71,72,75–77] in relation to an increasing amount of the necrotic tissue. Thus, in Lewis lung carcinoma (3LL) peak levels and AUC of doxorubicin were higher when the tumour weighed less than one gram[66] about 7 days after transplantation than at 25 days when about half, by weight, was necrotic. Doxorubicin was measurable in the vegetating part, but not in the necrotic portion. Similar results were obtained with methotrexate, methylnitrosourea and hypericin.[66,78]

Simulation of solid tumours *in silico* indicates that the core of the tumour contains large areas where drug concentrations are too low to have a cytotoxic effect.[79] However, no extrapolations are possible among different tumours or even different lines of the same tumour. Too many factors play a role in the delivery of anticancer agents from the blood to the tumour.[80]

Since the blood flow in tumours is usually lower than in normal tissues[81] attempts have been made to enhance it. Angiotensin II, by increasing the blood flow,[82,83] potentiated the efficacy of mitomycin C in experimental hepatoma[82] and doubled the intratumoural concentration of neocazinostatin compared to control rats.[84]

As summarised in a recent review,[85] besides the heterogeneity of the tumoural vasculature which contributes to uneven distribution of drugs even within the same tumour,[86] there is also poor lymphatic drainage[87] which, however, is important only for reducing the clearance of macromolecules[88] but negligible for small molecules that are redistributed into the circulation.[89]

10. Interstitial matrix

The low clearance of tumour macromolecules from the interstitium causes the interstitial fluid pressure (IFP) to rise, inhibiting the transvascular transport of drugs by blocking blood flow and leading to areas of necrosis.[80,90,91] The propensity of drugs to reach cancer cells is inversely proportional to the intercapillary distance, which increases with tumour size,[77,92] and to drug binding to cellular macromolecules, particularly in the extracellular matrix where the presence of collagen contributes to resistance to drug transport in the interstitium.[93]

An overview of the penetration of various anticancer drugs in spheroids and multicellular layers has recently appeared.[94] Of considerable interest is the possibility of increasing drug uptake in tumours by lowering IFP by cytokine inhibitors such as VEGF, PDGF, TNF and bradykinin (for a review see Ref. [95]).

Drugs move from the interstitium to enter cancer cells. There too, several factors are important: the physico-chemical characteristics of drugs, the presence of membrane transport systems, the cancer cells' ability to extrude xenobiotics, and the intracellular transport that allows drug distribution in the nucleus or in different organelles. Due to the lack of lymphatic drainage and leakage from tumour blood vessels, drugs with a molecular weight higher than 40 KDa may accumulate and remain a long time in a tumour, a phenomenon known as EPR (enhanced permeability and retention).[88] EPR can be increased by agents that inhibit bradykinin degradation[96] such as angiotensin-converting enzyme inhibitors (ACE-1). This causes accumulation of macromolecules in tumours.[69]

11. Three-dimensional tumours *in vitro*

To study drug penetration in the tumour, several *in vitro* methods have been proposed. In a system of two chambers separated by a collagen-coated microporous membrane, and cancer cells, drugs were added in the top chamber and measured in the bottom one.[97,98] Histocultures are fragments of tissue (1 cu-cm) from a tumour *in vivo*,[99–101] maintained on a collagen matrix. In this system drug penetration can be studied using labelled anticancer agents. Spheroids are multicellular three-dimensional agglomerates of cancer cells with different diameters (50–500 μm).[102–106] Drugs are analysed in the different layers. These methods consistently indicate that the penetration of almost all anticancer agents into solid tumours is very slow and frequently incomplete. Various tumours have been used including human xenografts. In general, uptake on the surface layer was very rapid but after that penetration was slow and did not reach the core of the spheroid.

The poor penetration of anticancer drugs has been shown, among others, for methotrexate,[104,107] vinblastine,[108] doxorubicin,[109,110] daunomycin,[111] actinomycin D,[111] cytosine arabinoside,[111] and paclitaxel.[99] Exceptions to this rule are 5-fluorouracil[108] and cisplatinum[112] although for 5-FU there is still a difference from its penetration in monolayers.[113]

To evaluate the potency of anticancer drugs it is interesting quantitatively to compare the penetration of anticancer agents in three-dimensional systems with the monolayer tissue cultures currently used. When doxorubicin was incubated with spheroids 150 μm in diameter, the drug was detected homogeneously throughout the cancer tissue. However, the drug penetrated only about 70–80 μm into spheroids 350 μm in diameter.[110] Doxorubicin's penetration of a three-dimensional tumour was 5–10 times slower than in monolayer cultures.[99]

In cultures of human pharynx xenograft tumours a steady-state concentration of paclitaxel was reached in about 48 h, as opposed to 4 h for monolayer cultures of the same tumour.[99] This slow penetration is ascribed to the high cell density of the tumour. Indeed, with higher doses of paclitaxel the penetration increased because of the apoptotic effect and a consequent decrease in the density of the tumour.[114] For these reasons human breast carcinoma spheroids were less sensitive to paclitaxel (14.33 ± 4.51 μM) than monolayers (0.15 ± 0.09 μM) after 1 h exposure, the difference decreasing with 24 h exposure.[115]

In human bladder cancer spheroids, vincristine was considerably less active than on the same cells cultivated as monolayers. The reason is not only the poor penetration of the drug but also the smaller number of G2+M cells in spheroids than monolayers.[106] Spheroids are probably a better

model to simulate solid tumours *in vitro* and should be preferred to tissue culture cancer cells.

12. Drug metabolism in tumours

Relatively little attention has been paid to the possibility of neoplastic tissue containing cytochrome P450 enzymes capable of metabolising antitumoural drugs that enter cancer cells. The importance of these enzymes may differ widely for antitumour drugs that are metabolised to active or inactive metabolites. For instance, in the case of cyclophosphamide and ifosfamide cytochrome P450 enzymes, they cause either activation through 4-hydroxylation (CYP3A4 and CYP2C9) or inactivation through N-de-chloroethylation (CYP3A4 and CYP2B6). A recent study using Western blotting in microsomal preparations from breast carcinomas showed the presence of CYP3A4 but not CYP3A5 and low positivity for CYP2C9 but not CYP2B6.[116] The significance of these findings in the overall formation of active metabolites remains to be established, but the possibility of direct metabolic activation in cancer cells remains attractive.

It is well known that several anti-metabolites with anticancer activity require intracellular metabolism. Cytarabine (ara-C) is phosphorylated to form 5′-ara CMP and then ara-CTP[117,118] which is incorporated into DNA.[119] Cladribine is also metabolised to 5′-triphosphate which inhibits DNA synthesis[120] and alters mitochondrial function.[121]

13. Intracellular localisation

The intracellular distribution of anticancer agents is sometimes important to achieve the binding with the target. Several agents including actinomycin D and doxorubicin interact with DNA and/or DNA-binding proteins and therefore must reach the nucleus.[122] In addition, doxorubicin shows an intra-mitochondrial localisation in the heart[123] which may explain its cardiac toxicity.

Since coupling anticancer drugs to macromolecules[124] or incorporating them into liposomes[125] seems to increase their accumulation in solid tumours because of the enhanced permeability and fenestration of tumoural vessels, several attempts have been made to develop different preparations.[126,127] For instance, doxorubicin incorporated into pegylated liposomes (DPL) is more effective than free doxorubicin in several animal tumour models.[128] It was therefore of interest to compare the intracellular distribution of DPL and free doxorubicin in lung cancer cells using confocal laser scanning microscopy. At the beginning of incubation (4 h) free doxorubicin concentrated in the nucleus while DPL was mainly in cytoplasm. After 24 h the fluorescence of doxorubicin shifted partly to the mitochondria and the Golgi apparatus while DPL reached the nucleus and was associated with the mitochondria but not the Golgi apparatus. Interestingly, neither drug was present in the lysosomal fraction.[129]

A whole class of chemicals known as delocalised lipophilic cations (DLC) is able to accumulate in mitochondria of cancer cells. This class includes rhodamine 123,[130,131] dequalinium,[132,133] ditercalinium,[134] thiacarbocyanines,[135] tetraphenylphosphorium[136] and a tellurium-containing cyanine.[137] The rhodacyanine (MKT077) has the advantage of being taken up by cancer cells 20–65 times more efficiently than by non-neoplastic cells.[138] This might be related to differences in mitochondrial membrane potentials.[139,140] Temoporfirin, an anticancer photodynamic agent[141] also accumulates in mitochondria of cancer cells.[142]

Studies on the intracellular localisation of anticancer agents are essential not only to understand mechanisms of action but also to design new drugs or preparations with greater ability to enter solid tumours and act on targets that are important for proliferation and dissemination of cancer cells.

14. Blood-brain barrier (BBB)

Many drugs are not available to the brain tissue and therefore also to tumours growing in the brain because of the BBB formed by the endothelial cells of the capillaries in the brain and astrocytes surrounding the capillaries.[143] The barrier is active because of tight junctions between endothelial cells[144,145] and limited transport due to endocytic activity and the absence of fenestrations. Antitumoural drugs can therefore only enter the brain by passive diffusion, therefore mostly compounds with small molecular weight and lipid solubility.[146] When drugs penetrate the brain their concentrations in cancer cells are fairly low though higher than in peritumoural tissue because of partial disruption of the barrier by the tumour.[147]

There are doubts that the relative resistance of brain tumours may be related to too low levels of anticancer agents. However, not all lipid-soluble drugs can gain access to the brain because the endothelial cells[148] have the drug efflux pump mentioned above encoded by one of the MDR (multidrug resistance) genes.[149] The importance of this pump is shown by the fact that PgP knock-out mice have substantial, long-lasting increases in their brain concentrations of several drugs, including doxorubicin,[150] vinblastine,[151] paclitaxel,[152] and docetaxel.[153] However, not all PgP inhibitors are effective in enhancing the penetration of antitumoural drugs in the brain, as shown by the case of vinblastine in normal mice.[154] Thus, the use of PgP inhibitors such as verapamil[155] did not result in substantial advantages for patients.[156] More recent studies have instead shown accumulation of paclitaxel in the brain with other more potent PgP inhibitors such as elacridal and valspodar.[152]

Imatinib mesylate is a potent inhibitor of the growth of glioblastoma *in vitro* and *in vivo*.[157,158] However, the drug penetrates human cerebrospinal fluid very poorly,[159] limiting its antitumour efficacy. The poor penetration is due to the interaction of imatinib not only with PgP but also with another transporter in brain vessel endothelial cells known as BCRP (breast cancer resistant protein).[160–162] Knock-out mice for PgP or BCRP, and the use of inhibitors of both transporter proteins do in fact increase the concentration of imatinib in brain.[163]

Pharmacokinetic studies help us understand that one of the difficulties in treating primary or metastatic brain tumours is obtaining adequate brain concentrations of antitumour

drugs. A number of attempts to modulate the BBB has been recently reviewed.[164]

15. Pharmacokinetics in primary and metastatic tumours

Metastases are the principal reason for tumour malignancy and the primary target of cancer chemotherapy. Since at the time of an early diagnosis metastases are relatively small in relation to the primary tumour, they should be fairly permeable to anticancer agents. In a mouse model of 3LL, 25 days after drug transplantation[165] doxorubicin and daunorubicin were present in lung metastases at concentrations about two and six times higher than in the primary tumour. Similar results were obtained for hydroxyurea, cyclophosphamide and methylnitrosourea.[66] These findings are in agreement with the observation that lung metastases have a higher blood flow than the primary tumour.[166]

In ovary cancer patients too, altretamine was concentrated several times more in pelvic and omentum metastases than in the primary tumour, although there was wide variability. Metastases less than 3 mm in diameter had significantly higher altretamine concentrations than larger metastases.[167]

However, metastases can occur in every part of the body, so the same anticancer agent may reach different concentrations according to the site. In a model of intratibial sarcoma 180, doxorubicin was not measurable in the primary tumour but concentrations were very high in renal and iliac metastases. In Walker carcinosarcoma transplanted intramuscularly in rats, doxorubicin AUC in lymph node metastases was twice that in the primary tumour. Methotrexate concentrations were two times higher in renal metastases than in the primary tumour. However, 6-mercaptopurine reached about the same concentrations in the primary and in the iliac metastatic tumour.[66]

While these findings may be taken as generally valid for small molecules there is a report that macromolecules, e.g. monoclonal antibodies, localised very poorly in lung metastases induced in guinea pigs by intravenous administration of bile duct carcinoma. The labelled monoclonal antibodies could not reach the internal layers of the metastatic nodules[168] even when injected at high doses. It is unfortunate that drugs are measured only rarely in metastases of experimental and human tumours.

16. Efflux and uptake pump proteins – ABC transporters

Besides being present in normal tissues (in particular brain and testis) a number of tumours involve the presence, spontaneous or inducible, of efflux pump proteins that in several cases limit the availability and the consequent efficacy of antitumoural drugs.[24,169,170]

The PgP encoded by the multidrug resistance gene MDR-1 is the best known member of a family of ATP binding cassette (ABCC) transporters. There are at least two other ABCC transporters associated with drug resistance, the multidrug resistance protein (MRP1),[171] and the mitoxantrone resistance protein (MR/BCRP).[172] The MRP family contains at least six members.[24,25] There is ample information about the association of MDR or MRP with reduced entrance of antitumoural agents:[173] this impairment can be reversed by the use of a number of MDR or MRP expression inhibitors.[14] A novel way to knock out PgP uses a non-viral SB based RNA transposon vector system for the stable breakdown of MDR1, which lasted several months. Chronic myeloid leukemic K562 cells resistant to doxorubicin or to imatinib when treated with this vector lost the efflux of anticancer agents and regained sensitivity to doxorubicin or imatinib.[174]

Increased entrance of drugs with a reversal of the sensitivity of cancer cells indicates a causal relationship between the ABC transporters and the sensitivity of cancer cells. Unfortunately, the problem of overcoming drug resistance by changing the entrance of antitumoural drugs into cancer cells is very complicated on account of the heterogeneity among tumours and within a single tumour, which can cause uneven drug distribution.[175,176]

For instance, an analogue of cyclosporine A, PSC 833, in mice bearing leukaemia P388, raises doxorubicin concentrations about two-fold in sensitive leukaemic cells, and about seven-fold in leukaemic cells resistant to doxorubicin. However, the line of leukaemia P388 resistant to doxorubicin *in vivo* was not reversed to a sensitive one, suggesting other mechanisms of resistance.[14] In addition, PgP may be difficult to detect due to epitope masking by sialic acid.[177] Polymorphism of MDR has also been reported.[178] The members of ABC transporter family are described in detail in reviews.[24,25,170]

17. Nucleoside transporters

Nucleoside anticancer drugs such as cytarabine, fludarabine, or cladribine require the use of nucleoside transporters (NT) which physiologically mediate the uptake of purine and pyrimidine nucleosides.[179] There are two classes of NT in human cells and tissues, the equilibrative nucleoside transporters (ENTs), with four functional subtypes,[180] and the concentrative nucleoside transporters (CNTs) with six.[181] hENT1 and hENT2, the best characterised proteins are widely distributed in tissues.[182] hCNT1-3 are present in several tissues including intestine and kidney.[183,184] NTs are also seen in several types of cancer cells but usually less than in the corresponding normal tissues,[183] ENTs being less expressed than CNTs.[182]

Several antimetabolites have to be taken up in order to enter cancer cells, as shown by their different efficacy on cells with high or low expression of NTs. The inhibition of hENT1 by dipyridamole makes cancer cells resistant to gemcitabine.[185] The hENT1 abundance in infants with acute lymphoblastic leukaemia has been considered the reason why cytarabine is more active than in older children.[186]

However, the problem is much more complicated because the expression of NT proteins is only one of many factors in anticancer drug efficacy. NT polymorphism may be important[187] but a recent attempt to correlate NT expression in various cancer cell lines to their sensitivity or resistance was inconclusive.[188]

18. Lessons from pharmacokinetic studies of anticancer agents

Pharmacokinetics, including absorption, distribution, metabolism and excretion, must be considered an important part in the mechanism of action of drugs and in pre-clinical and clinical development of new drugs. As we hope we have shown in this mini-review, this is also true for anticancer agents, particularly at present when a large number of potentially new drugs, small and macro molecules, conventional and targeted agents are available for clinical trials.

It is clear that cancer tissue culture *in vitro* must be combined with other *in vitro* tests because the persistence of drugs in the medium, the limited metabolism and the diffusion into the cancer cell monolayer can give an overoptimistic evaluation of the potency of anticancer drugs. The frequently cited equation, that the EC50 *in vitro* must be reproduced in the concentrations measurable in serum after *in vivo* administration is no longer tenable.

Techniques such as spheroid or histocultures must become part of routine evaluation *in vitro* to establish the penetration of anticancer agents and hence their efficacy in a system that mimics the conditions of solid tumours *in vivo*.

New complex *in vitro* systems as well as better characterisation of the tools available are needed. The concentrations of anticancer agents in a three-dimensional model and their distribution in the different layers may give important hints to facilitate subsequent testing of the drug.

Pharmacokinetic studies *in vivo* should be seen not only as a burden required by the regulatory authorities but as a way to find out whether a drug is distributed preferentially to some tissues and to compare the concentrations in the tumour and normal tissue. This information may be very important to predict specificity and/or toxicity of anticancer drugs.

The solid tumours used in experimental research also need to be characterised better in terms of their vascularity, matrix composition, presence of metabolising enzymes and ABC and NT transporter proteins, to correlate these parameters with the extra and intracellular concentrations of anticancer drugs in the vegetating and the necrotic areas. Tumours must be transplanted in areas orthotopic to their origin to take advantage of a preferential distribution of anticancer drugs. More attention should be paid to tumours that metastasize in different organs because metastases are the essence of malignancy. Drug concentrations in metastases must also be measured to predict the action of anticancer agents in advanced human cancers.

Efforts to boost the selectivity of anticancer drugs and make them 'intelligent' must be complemented with pharmacokinetic knowledge. Obviously if a drug does not reach its target in the complex *in vivo* situation it cannot be effective, but also if a drug is present but is bound to blood or tissue protein, or is rapidly metabolised, it cannot be effective. Metabolism is an important part of drug action because metabolites can sometimes be the active principles. The example of cyclophosphamide or irinotecan must always be borne in mind and this may lead, at least in suitable cases, to measuring drug efficacy *in vitro* in the presence of liver microsomal fraction containing cytochrome P450.

The failure or the limited activity of so many anticancer agents in patients should make researchers multiply their efforts at the preclinical level so as to better select the tumours with the best chances of being sensitive to new anticancer agents. In patients more efforts should be made in the various experimental phases to establish the variability of serum concentrations in order to select patients better. With due ethical considerations, more studies are needed on surgical specimens of the primary tumour and/or metastases to measure the levels and distribution of anticancer agents, to check for correlations with the tumour characteristics and for associations with the outcome of treatment in single patients. These considerations are not in opposition but must be seen as a necessary complement to current efforts to establish genomic profiles of the host and the tumour because the phenotypic and genotypic aspects must in the end be integrated.

In the fight against cancer all the scientific knowledge and techniques of pharmacokinetics must be put to good use. The abundance of new approaches to find anticancer drugs calls for closer critical evaluation than in the past to select drugs with the best probabilities of being effective in patients with tumours. Ethical and scientific reasons dictate that patients must not be exploited and resources must not be dispersed by testing drugs poorly characterised.

Conflict of interest statement

None declared.

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