Pergamon

European Journal of Cancer Vol. 31A, Suppl. 7, pp. S18–S20, 1995
Elsevier Science Ltd
Printed in Great Britain
0959–8049/95 $9.50+0.00

0959-8049(95)00294-4

Pharmacokinetics, Targeting and Delivery Systems in Anthracycline-resistant Cancers

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INTRODUCTION

BREAST CANCER is generally considered to be a chemosensitive tumour. This is particularly so when the patient is first exposed to chemotherapy. However, as clinicians will be aware, the majority of patients will eventually display relative resistance to chemotherapy. The mechanisms for this derived resistance are multiple, and are discussed in detail in other manuscripts from the ESO Task Force Workshop. Even at the first exposure to chemotherapy, not all patients will respond to anthracycline treatment. Does this indicate *de novo* resistance? The answer must be qualified. What was the dose and schedule used, of which anthracycline? Did the patient experience side-effects of therapy? Is there reason to believe that the patient may handle the drug differently from other patients (e.g. hepatic dysfunction)?

It is well known that plasma pharmacokinetic parameters display wide variability, even in patients given a 'standard' dose and schedule of an anthracycline. The plasma area under the concentration–time curve (AUC) may vary by as much as 10-fold for doxorubicin. If one then considers that each individual will have different sites and bulk of metastatic disease, with consequent differences in vascularity, oxygenation and drug penetration, it is little wonder that some patients appear to be resistant to the standard dose. This is likely to be independent of any cellular mechanisms of drug resistance and is known as 'pharmacological resistance'. In other words, the hypothesis is that if sufficient concentration could be delivered to the site of action, for a sufficient duration, then the tumour would have responded to that agent.

Are there data to support this hypothesis? The burgeoning field of high dose chemotherapy is built on the evidence that 'more is better'. I will not review this issue [1], but would point out that some studies which purport to confirm this rationale actually demonstrate that low dose therapy is inferior to standard dose therapy. An example is the Glasgow study of high (100 mg/m^2) versus low (50 mg/m^2) dose epirubicin in advanced breast cancer [2]. This randomised prospective trial demonstrated that 100 mg/m^2 had a significantly superior response rate (41%), with more toxicity. Does this confirm that 'more is better'? A different interpretation is that 50 mg/m^2 (RR 23%) is inactive, inadequate treatment. To take this to an extreme, what response rates could be expected in a 10 mg/m^2 versus 100 mg/m^2 study? A more appropriate design to support high dose therapy is to compare standard dose with escalated dose.

In tissue culture, it is simple to demonstrate anthracycline dose–response against breast cancer cell lines. For the reasons given above, it is not clear that a clinical dose–response can be assumed. If we remove the variability in handling by considering only the plasma AUC versus response, the correlation improves but is far from perfect [3]. If we consider the tumour concentration of anthracycline—does this correlate with response? The literature is somewhat conflicting on this issue, as illustrated by two small Glasgow studies based on HPLC (high performance liquid chromatography) measurement of drug in tumours. The first study [4] claimed that in animal tumour models the accumulation of doxorubicin was closely correlated with tumour responsiveness, the second [5] investigated tumour concentration in patients with breast cancer. In this case, no relationship could be found between anthracycline content in tumour and treatment outcome.

Other reports in the literature use fluorescence as a method of quantification of anthracycline uptake, but since this methodology is confounded by quenching of fluorescene when anthracyclines interact with DNA, the conclusions are also suspect. Even the HPLC methodology has experimental limitations which may partially explain the lack of consensus. The stromal component of the tumour may be important, accumulating drug at a different rate from the tumour cells. Alternatively, biopsy material may not be representative of the tumour as a whole and, finally, individual tumour cells may have intrinsic variation in drug accumulation or sensitivity to the cytotoxic actions. Even at the level of multicellular tumour spheroids, it is clear that penetration of anthracyclines remains a problem [6]. It is, therefore, not surprising that clinical dose—response data are so difficult to generate.

DRUG DELIVERY

Despite lack of substantiative evidence that 'more is better', it would be counter-intuitive to postulate that more drug in the tumour would lead to a reduction in response. In addition, we know that if less drug reaches the organs of toxicity, less toxicity will be experienced by the patient [7, 8]. In the situation of advanced breast cancer, it would be reasonable to expect that optimum palliation could be achieved by maximising drug delivery to tumour sites, whilst minimising drug delivery to organs of toxicity.

How could we achieve this ideal situation? It may be possible by technique of adaptive feedback control, similar to those used in therapeutic drug monitoring for agents such as gentamicin, to optimise the individual dose and schedule for each patient [3]. In order to do this, we must have some idea of a 'target' drug concentration or pharmacodynamic parameter (e.g. nadir neutrophil count) which is associated with maximal response

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Table 1. Ideal requirements for a drug delivery system

— ability to incorporate a broad spectrum of drugs
— maintenance of activity of bound drug
— protection of drug from premature degradation
— non-immunogenic
— target recognition
— delivery of therapeutic drug concentrations
— release of active drugs at the target
— biodegradable
— biologically inert
— feasible large scale production
— cost-effective

or minimal acceptable toxicity. One possible pharmacokinetic parameter is the initial concentration constant A which Robert and colleagues [9] claim is associated with high tumour uptake of doxorubicin and enhanced therapeutic response. This strategy of dose optimisation for the individual is being prospectively tested in the clinical arena, and it remains to be seen whether it will be feasible and result in clinically meaningful improvements in therapy.

DRUG TARGETING

An alternative method of achieving the same objective would be to employ a targeting system. Over the last 20 years, a large number of systems have been proposed and, to varying degrees, have been developed. The remainder of this review will concentrate on those which have attracted the most scientific interest, and those which appear to offer therapeutic advantages.

Table 1 outlines the theorectically ideal requirements for a targeting system. Few, if any, of those currently available approach this ideal. Table 2 describes targeting specificity [10].

In addition, targeting may be passive, relying on the inherent properties of the delivery system; or it may be active with use of, for example, vasoconstriction [11], magnetic properties [12] or monoclonal antibodies.

It is worth noting that efficient drug targeting may overcome not only pharmacological resistance, but also be able to circumvent the MDR phenotype or other mechanisms of resistance, by presenting an excessive amount of drug which then overwhelms the resistance mechanism, or by presenting drug to an intracellular compartment which bypasses the resistance (i.e. third order targeting). In laboratory models of resistance, increased concentration of drug will eventually overcome resistance, but at concentrations which would be unrealistic in human practice.

MONOCLONAL ANTIBODIES

The application of antibodies to cancer therapy has been intensively investigated over the last 10–15 years. This has largely been driven by innovations in the production of monoclonal antibodies and hybridoma techniques, and more recently by the ability to genetically engineer antibody fragments and 'humanised' or chimaeric antibodies.

Table 2. Targeting strategies

First order:	to organs or tissues
Second order:	to specific cell types
Third order:	to selected intracellular compartments

Since antibodies are raised to single antigens, the potential exists for selective targeting to cells presenting the antigen. However, it is unusual, and may be impossible, to find a single antigen which is only expressed in tumour tissue, and is expressed in 100% of tumour cells, and is accessible to penetration of the antibody at the tumour site. These attributes are particularly important in cancer, since if even a small proportion of cells survive the cytotoxic therapy, this would be sufficient to repopulate the entire tumour. These problems, together with considerations of the antigenicity of the (mainly murine derived) monoclonals themselves, have somewhat dampened the high level of optimism which surrounded this approach in the early 1980s.

Limited success has been achieved in immunotoxin therapy of lymphoma, in which the problems of penetration are not as important [13]. The major focus of attention has now switched to a concept known as ADEPT (Antibody Directed Enzyme Producing Therapy). In brief, this involves the use of an enzyme antibody conjugate which selectively localises in tumour tissue. This is followed by administration of an inactive prodrug which is a substrate for enzyme. Thence, on enzymic cleavage of the prodrug, a cytotoxic species is liberated into the environment of the tumour cells. This approach was pioneered by Bagshawe [14] and is now in phase 1 development. In this case, the investigators use a bacterial carboxypeptidase G2 linked to an anticarcinoembryonic antigen antibody (CEA is expressed by many colon cancer cells), with an L-glutamic acid nitrogen mustard prodrug.

Optimistic results have also been published recently using a combination of monoclonals and liposomes, but as yet this has not reached clinical application [15]. A number of other interesting developments in this field were reported at the recent ASCO meeting in Dallas.

PARTICULATE SYSTEMS

A multitude of particulate systems have been tested. They vary in size, chemical composition, efficiency of delivery and release characteristics [16]. The major problems with such microparticulates are their stability and their sequestration by the reticulo-endothelial (RE) system. These factors generally limit their usefulness to locoregional therapies, including chemoembolisation. These limitations may be overcome by developments in polymer chemistry and a greater understanding of particulate–cell membrane interactions, but it is doubtful if particulate-based systems will make a major contribution to cancer therapy in the near future.

LIPOSOMES

Liposomes are uni- or multilamellar vesicles which enclose a fluid compartment. The drug can associate with the aqueous or the lipid phase, allowing delivery of some agents with poor inherent aqueous solubility. They are attractive as potential carrier systems for a number of reasons [16, 17]. Their composition and size can be easily modified for different applications, their degradation products are non-toxic, drug release characteristics can be tailor-made, and they may be conjugated with other molecules allowing a degree of active targeting [15]. Initial development in the early 1970s was greatly hindered by the problems of reproducibility, due to impure phospholipids, and

due to RE trapping [18]. Various techniques have been employed to try to circumvent RE accumulation, the most successful being the inclusion (in the lipid composition) of negatively charged components which confer long circulation time and increased stability. This is the basis of so-called 'stealth' liposomes ('stealth' is a registered trademark of Liposome Technology Inc., Menlo Park, California). In comparative studies with 'conventional' liposomes, this version has a number of advantages [19].

Clinical trials of conventional and 'stealth' liposomes have been performed. In general, these trials show a reduction in toxicity, allowing escalation of the maximal tolerated dose of doxorubicin to 120 mg/m^2 every 3 weeks, with alterations in pharmacokinetics compared to the parent drug [20]. Not surprisingly, due to the profile of patients enrolled in phase 1 studies, antitumour response has been limited.

It is conceivable that other modalities of pharmacokinetic modification of anthracyclines, such as prolonged infusion, could result in a similar alteration in toxicity and antitumour effect. However, the versatility and the simplicity of liposomes is significant, and it is likely that some of these systems will see clinical utility in oncology therapy.

POLYMERIC CARRIERS

The polymeric carriers, and in particular the anthracycline *N*-(2-hydroxypropyl) methacryamide copolymers (HPMA) described by Duncan and colleagues [21] appear to approach our criteria for an ideal system. These macromolecules can be tailored for molecular weight, drug content, drug linkage and biodegradability. They are non-immunogenic and highly effective in a number of anthracycline-resistant model systems [22, 23]. The first demonstration of *in vivo* activity with this system was in 1989 against a Walker sarcoma rodent tumour model with pharmacokinetic data confirming a higher tumour area under the concentration–time curve (AUC) and a reduction in cardiac peak levels and AUC [22]. Targeting may be passive through EPR (enhanced pentration and retention) due to leaky tumour vasculature, or it may be active by addition of targeting moieties distant from the drug. In this case, the polymer–drug linkage is also designed to be cleaved only in lysosomes within actively endocytosing cancer cells.

In animal model systems, acute toxicity and cardiac accumulation of anthracyclines are reduced [22], and a phase 1 study in cancer patients is currently underway under the auspices of the U.K. Cancer Research Campaign. Again, the potential for engineering the system to specific circumstances is significant.

CONCLUSIONS

It is likely that both 'pharmacological' and cellular resistance mechanisms are active in breast cancer. Perhaps the balance changes betwen the two i.e. acquired cellular resistance—as the tumour progresses, and selection pressures are applied by exposure to cytotoxins. The use of systems which divert more drug to the tumour and less to the organs of toxicity have shown more promise but, as yet, none have made a major practical impact. The potential for old drugs, used in optimum schedules, should not be forgotten in our desire to produce new therapies for patients with anthracycline-resistant breast cancer.

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