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The Chemotherapy of Colon Cancer

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Despite extensive clinical trials, mortality from colon cancer has remained essentially unchanged since the 1950s. However, the increasing numbers of complete and partial responses seen in clinical trials suggest that colon cancer can be successfully treated by chemotherapy, but only if the antitumour selectivity can be increased by a substantial amount. This will be possible by the introduction of new drugs with more precise mechanisms of action, such as those acting specifically on signalling or cell cycle control pathways shown to be aberrant in colon cancer. Alternatively, the selectivity of present day agents may be increased considerably by the selective activation of prodrugs in tumours (ADEPT) or by targeting them to tumours using polymers. Other new approaches using vaccines or some form of gene therapy will potentiate present chemotherapy, while the introduction of positron emission tomography (PET) scanning will allow the rapid detection of agents with activity that would have been missed by conventional measurements of response.

Key words: colon cancer, chemotherapy, antibody targeting, drug targeting, polymer targeting, prodrugs, PET scanning, vaccines

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INTRODUCTION

DEATH RATES from colon cancer, which were high in the 1920s in England and Wales, fell until the 1950s. Since that time there has been little change in mortality from cancer of the colon and rectum, which are responsible for 12% of all deaths from cancer. Since cancer chemotherapy only began to be practised on a large scale from the 1950s, this form of therapy has clearly had little impact on the mortality of colon cancer [1]. Each year the results of many new chemotherapy trials against colon cancer are reported. Some of the trials may involve agents with new mechanisms of action, for example, inhibitors of thymidylate synthase. Often they comprise more than one agent in new combinations, perhaps with cytokines and altered dose regimens, such as prolonged continuous infusions or intrahepatic arterial injection. Attempts have also been made to improve the selectivity of active agents, such as 5-fluorouracil, by biochemical modulation with, for example, folinic acid, *N*-(phosphonacetyl)-l-aspartate (PALA) or levamisole. However, while the response rates may be improved for metastatic cancer, there is no evidence that any of these treatments prolong overall survival. A reasonable conclusion would be that these present day agents are not selective enough to be curative treatments for metastatic colon cancer, and that a decrease in overall mortality from colon cancer will only be achieved if their selectivity can be increased by several orders of magnitude, or if new agents are introduced which are more specific in their anticancer action. Recent results have indicated that both these approaches may lead to new and more effective treatments for colon cancer.

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NEW CLASSES OF ANTICANCER AGENT

The past few years have seen the development and, in some cases, the clinical trials of chemicals that are quite different from antiproliferative agents in their mechanism of action. Among these are antiangiogenic agents, which may interfere with the formation of new blood vessels and inhibit tumour growth, antimetastatic agents, which may prevent the invasion and further spread of tumours, and chemicals which may induce apoptosis. Any of these might be used in future trials for the treatment of metastatic colon cancer. Impressive advances have been made in our knowledge of the intracellular mechanisms which regulate the progression of the cells through the cell cycle, and of the processes by which cells respond to external growth factors. The cell cycle is checked at various points by the transient association of an intracellular cyclin with a cyclin-dependent kinase. Only following specific phosphorylation/dephosphorylation and disassociation of the complex can the cell progress into the next stage of the division cycle. There is ample evidence that abnormal functioning of these checkpoints is associated with malignancy [2]. In some cancers, the cyclins appear to act as oncogenes, while they are overexpressed in others. Also, natural inhibitors of certain cyclins are thought to play a key role in the malignant behaviour of certain cancers [3]. The pathways by which growth factors initiate cell division by intracellular signalling mechanisms have also been elucidated in considerable detail, and again have shown that aberrant functioning of these pathways is associated with malignancy [4]. Thus, in tumour cells, there are quantitative differences of normal cells which may be exploited in drug design, and many potential antitumour agents are under development which act specifically on these aberrant pathways. Among these are inhibitors of growth factor binding, mitogenic peptide antagonists, G-protein inhibitors, modulators of protein kinase C and inhibitors of phospholipases, membrane calcium channels, protein tyrosine

kinase and of the inositol phosphate pathway. As more is learned about these pathways in human colon cancer, it will become apparent which of these new agents will be most appropriate for therapy, possibly as combinations of agents acting on different stages of the cell cycle or signalling pathway. Their development will be far more rapid than hitherto. The automated and combined use of chemical building blocks allows the synthesis of libraries containing vast numbers of potentially active analogues. These may be rapidly screened by high throughput cell- or receptor-based systems, and the use of such techniques has often produced lead chemicals within a matter of weeks.

Limonene, a chemical studied over many years because of its anticarcinogenic properties, is already on clinical trial because of its ability to inhibit the signalling of p21ras. *RAS* genes form a pro-ras protein which is then processed by a series of post-translational events. A carboxypeptidase removes the last three amino acids to leave a terminal cysteine which is then carboxymethylated and polyisoprenylated. Only at this stage can it be localised in the plasma membrane and take part in signalling [5]. Limonene, which has activity against chemically induced tumours which express constitutively activated *RAS* genes, inhibits the isoprenylation stage at a point in the mevalonate pathway distal to 3-hydroxy-3-methylglutaryl coenzyme A [6]. Since 50% of colon tumours express mutated *RAS*, they may prove to be susceptible to treatment with limonene. The clinical trial has reached the end of phase I, where responses have been observed at very high dose levels and phase II clinical trials are planned (C. Coombes, Charing Cross Hospital, U.K.). Any antitumour activity found in phase II trials will almost certainly lead to further studies in this area because limonene is not very potent and is extensively metabolised *in vivo*. Further, two of its metabolites are more potent inhibitors of the mevalonate pathway [6]. Other inhibitors of the mevalonate pathway are known including peptides and polyisoprenoids and could be optimised by analogue development using synthetic combinatorial libraries described above.

PRODRUGS AND TARGETING

Studies over the years have established that the higher the total dose of cytotoxic agents that can be given, the greater the tumour response. Detailed trials on dose intensification have concluded that for the more resistant solid cancers, such as metastatic colon cancer, cures would be achievable, but only if dose levels 50–100-fold those of conventional doses could be administered. Procedures, such as local intrahepatic arterial injection, the use of normal stem cell protectors or growth stimulators and autologous bone marrow transplants, may allow the administration of higher dose levels. Unfortunately, they are nowhere near the 50–100-fold increase required for total tumour stem cell eradication. However, prodrugs can easily increase by 50-fold the amount of drug delivered selectively to the tumour. Prodrugs have many uses in medicine, but in cancer therapy (since the aim is to eradicate all tumour stem cells), the prodrugs used are non-toxic chemicals that can undergo enzymatic conversion to metabolites that may be many orders of magnitude more toxic than the prodrug. For selectivity to be achieved, the activation must only take place in the tumour environment, and hence the activating enzyme must be unique to the tumour or expressed at very high levels compared to normal tissues. Figure 1 shows the enormous selectivity theoretically achievable with the appropriate use of prodrugs. CB1954 is a non-toxic prodrug which is converted by the enzyme DT-diaphorase into a highly toxic bifunctional alkylating agent. As a consequence, cells that express the enzyme are more than 10 000 times more sensitive to the prodrug [7]. Similar results have been obtained *in vivo* where animals with transplanted tumours insensitive to chemotherapy are cured by prodrugs, even when there are extensive metastases [8]. Preliminary clinical trials concluded that, although human tumours expressing an appropriate enzyme are sensitive to prodrug therapy, such tumours were rare and unpredictable, that is, they were not associated with any particular tumour type. Furthermore, it has now been shown that there may be large differences between the activity of rodent enzymes which were used in the development of prodrugs and the corresponding human enzymes. Because of the need to biopsy tumours to determine their enzyme content, it was not feasible as a routine procedure. The approach was revitalised in the 1980s with the demonstration that certain monoclonal antibodies are strongly tumour-associated. Some monoclonal antibodies raised against carcinoembryonic antigen (CEA), for example, bind strongly to metastatic colon cancer but only weakly to a few types of normal cell. Bagshawe suggested that the clinical problem found with prodrug therapy, namely the scarcity of tumours expressing a unique activating enzyme, could be remedied because tumour-associated antibodies could be linked to non-mammalian enzymes and directed to the metastasis, which would thus acquire an environment high in a unique enzyme capable of activating a prodrug [9]. The procedure has been termed ADEPT (antibody-directed enzyme prodrug therapy). As shown in Figure 2, an antibody–enzyme conjugate is administered which binds to malignant cells. Because of the catalytic properties of enzymes, relatively few molecules of the conjugate need to be bound because a single enzyme molecule may activate many prodrug molecules if K_{cat} (turnover rate) of the enzyme for the prodrug is high. Also, not all tumour cells need to express the antigen since released drug can diffuse to neighbouring cells that do not express the antigen. Antibodies for different types of human tumour (lung, colon, breast, ovary and choriocarcinoma) have been linked to a variety of enzymes (including human enzymes) and used in human tumour xenograft experiments. Prodrugs of toxins and several types of anticancer agent have been investigated, and regressions and cures have been obtained in many different classes of

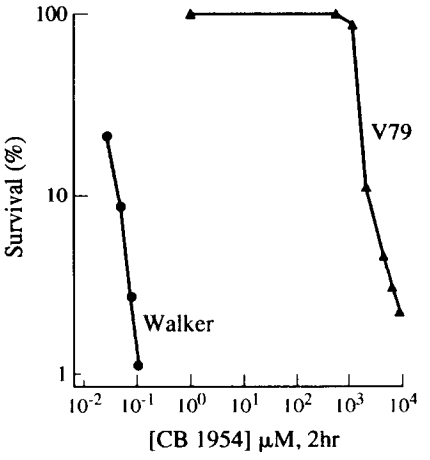

Figure 1. The effect of CB1954 on the colony-forming ability of Walker cells or Chinese hamster V79 cells. All treatments were for 2 h at 37°C. The concentration of CB1954 is plotted on a logarithmic scale. Whilst Walker cells have high levels of the activating enzyme DT diaphorase, V79 cells have very low levels.

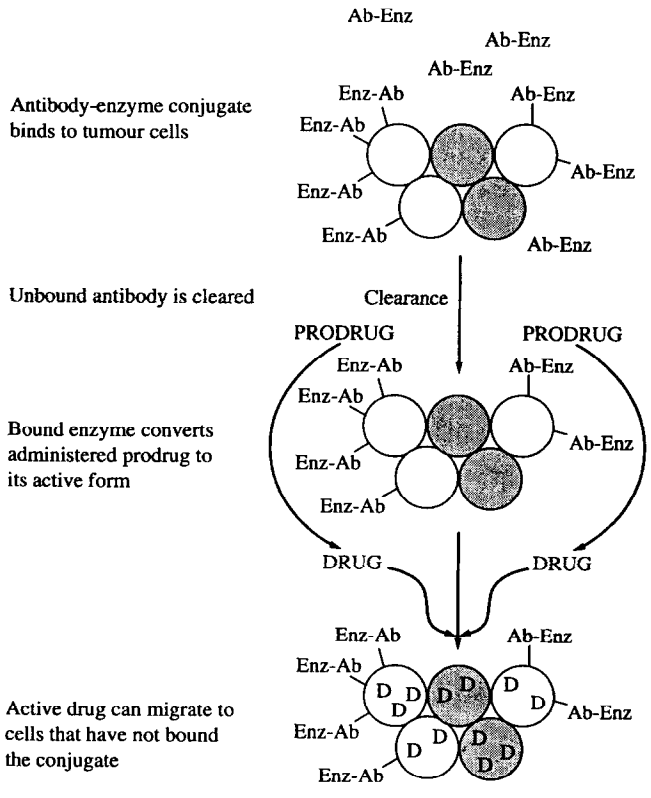

Figure 2. The generation of a cytotoxic drug by ADEPT. In the first phase, the antibody–enzyme conjugate is allowed to bind to the target cell population. After unbound conjugate is allowed to clear, a prodrug is administered which is converted to an active drug (D) by the bound enzyme. Importantly, the active drug can migrate and have effects on cells that have not bound the conjugate.

tumour, most of which are resistant to conventional anticancer agents [10]. The first clinical trials have been against metastatic colon cancer using the bacterial enzyme carboxypeptidase G2 linked to an antibody fragment raised against CEA (R. Begent, Royal Free Hospital, U.K.). Tumour responses have been observed in these early trials and further trials are planned using more appropriate prodrugs.

TARGETING BY MEANS OF POLYMERS

A promising alternative for treatment of colon cancer including metastatic deposits is the relatively new concept of polymer drug conjugates [11,12]. Conjugation of an antitumour agent to a polymeric carrier creates a macromolecular prodrug whose molecular weight can be tailored to optimise pharmacokinetics. The molecule can incorporate drug–polymer linkers (for example, peptides and pH-sensitive spacers) selected to promote preferential and prolonged intratumoral drug release. Polymer–cytotoxic drug conjugates are now in the early stages of drug development and are already showing considerable promise.

N-(2-Hydroxypropyl)methacrylamide (HPMA) copolymer–drug conjugates containing either anthracycline antibiotics [13] or alkylating agents [14] bound to polymer through peptide linkers designed for cleavage by thiol-dependent enzymes (cathepsins B, H, L) have been described [11]. It has been shown that drug conjugation creates a product with decreased toxicity and increased efficacy *in vivo* [13, 14]. Currently, HPMA copolymer doxorubicin (molecular weight ~25 000; doxorubicin content ~10% of total weight) containing a Gly-Phe-Leu-Gly spacer is in phase I clinical trials (Figure 3). This compound displays impressive antitumour activity *in vivo*, particularly against solid tumour models [13, 15, 16] (Figure 3). In addition, covalent conjugation leads to a marked reduction in doxorubicin-related toxicity (three to five times less toxic), including a marked reduction in cardiotoxicity [17].

Tumour-specific drug deposition is an important consequence of transformation of a low molecular weight antitumour agent into a polymeric drug. Using a number of *in vivo* solid tumour models (Walker carcinoma, B16 melanoma and sarcoma 180), it has been clearly demonstrated that HPMA–copolymer conjugates [16, 18, 19], like other molecules with prolonged plasma residence times, can accumulate preferentially due to a phenomenon that has been called by Maeda and colleagues the enhanced permeability and retention effect (EPR) [20]. The EPR is a consequence of the increased permeability of the tumour vascular endothelium, which allows extravasation of circulating macromolecules that do not readily escape into most normal tissues. Inherent lack of tumour lymphatic drainage prevents rapid clearance and ensures that the macromolecule concentration rises significantly. Levels of HPMA copolymer doxorubicin (at a dose of 5 mg/kg doxorubicin equivalent) detected in the subcutaneous B16 melanoma were more than 15-fold higher than free doxorubicin administered at the same dose (Figure 4). A good correlation was shown with antitumour activity [15]. Although HPMA copolymers of molecular weight 20 000–30 000 display prolonged plasma residence times compared to free drug, they are small enough to be excreted relatively quickly due to glomerular filtration ($T_{\frac{1}{2}}$ approximately 1 h [21]). It is clear that optimisation by increasing molecular weight provides the opportunity to elevate still further the intratumoral level of drug conjugate [19].

HPMA copolymer doxorubicin displays an increased therapeutic index (compared with doxorubicin) in the human colorectal xenograft LS174T model (Figure 3) [13] and was more effective than either free 5-fluorouracil (5-FU) or polymeric matrices containing 5-FU in the same model [22]. Due to the EPR effect, it seems probable that HPMA copolymer constructs will selectively localise in primary and metastatic tumours of many different types, including colon cancer, and thus by the synthesis of the appropriate covalent conjugates, they have the potential to extend the spectrum of activity seen with currently used anticancer agents. Additionally, they should improve the tumour specificity of novel agents (e.g. tyrosine kinase inhibitors) and strategies (gene and antisense therapies) presently under development.

Inclusion of organ- and tumour-specific targeting moieties into polymer structures provides the opportunity to target antitumour agents to the vicinity of, or directly within, tumour cells. One system extensively studied relies on the inclusion of galactosamine residues in the copolymer structure [23] to provide liver-specific delivery through the hepatocyte asialoglycoprotein receptor. This approach leads to liver deposition of a substantial proportion of an intravenous dose (approximately 70%) [23] if care is taken not to saturate the receptors available for selective uptake by administration of a high bolus dose [24]. Liver-targeted doxorubicin has the potential to treat liver metastases because the high concentration of the drug in the liver should access tumour cells via the bystander effect. HPMA copolymer doxorubicin–galactosamine will enter phase I trials in a year or so. The polymer structure can be modified to permit radiolabelling, and gamma camera imaging of HPMA copolymer conjugates (containing doxorubicin with or without galactose) has

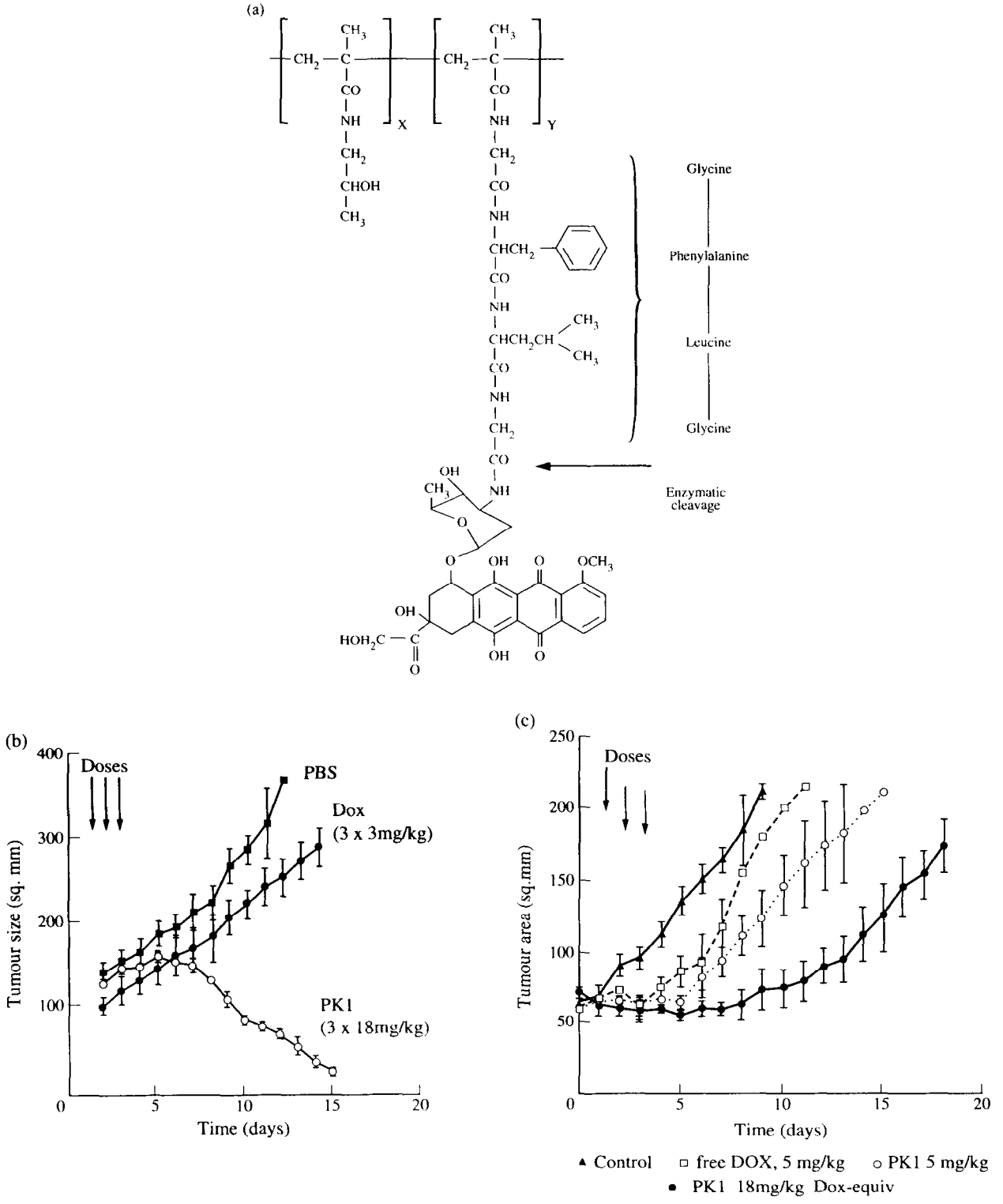

Figure 3. Structure and antitumour action of HPMA copolymer doxorubicin. (a) Structure. (b) Activity against subcutaneous P388. (c) Activity against the human colorectal xenograft L5174T.

been reported in mice [25]. Imaging is currently being used in early clinical studies, both to monitor body distribution and to assist the evolution of dosing schedules that will allow optimisation of receptor targeting in humans.

Polymer conjugates are still a little known concept in medicine. The last 15 years, however, have seen much elegant experimentation, and pioneering industrial development has already successfully transferred two polymer–protein conjugates into routine clinical use. Polyethyleneglycol (PEG) L-asparaginase [26] and styrene-maleic anhydride-neocarzinostatin (SMANCS, Zinostatin, Stimalmer) [27] have been approved by the regulatory authorities in the U.S.A. and Japan, respectively, for use in the treatment of acute lymphocytic leukaemia and primary hepatocellular carcinoma (intrahepatic artery administration). These are the first novel drug delivery systems (including antibody-based and liposomal systems) to success-

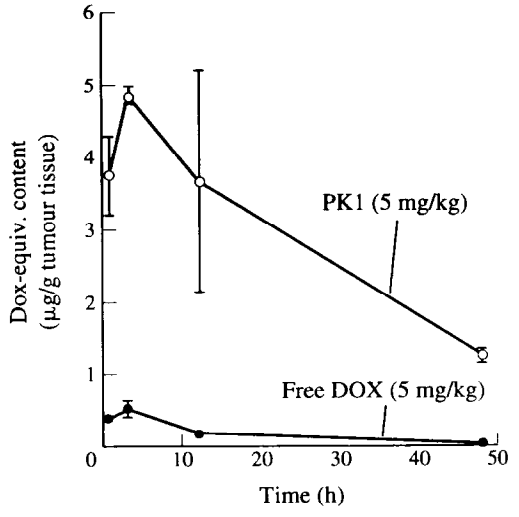

Figure 4. Accumulation of doxorubicin and HPMA copolymer doxorubicin in subcutaneous B16 melanoma. Free drug and polymer-bound drug were quantitated by HPLC after an intravenous injection.

fully complete a clinical development programme for the treatment of cancer, reinforcing the belief that polymer constructs are amenable to design as improved anticancer therapies, and that they can overcome the rigours of industrial development.

OTHER APPROACHES

Many new trials of vaccines have been reported, some of which may be used in the treatment of metastatic colon cancer. These include an anti-idiotype vaccine that mimics an epitope of a tumour-associated antigen, a vaccine made of autologous colon cancer cells infected with Newcastle disease virus and cells genetically modified to enhance their immunogenicity [28, 29]. Hepatic metastases would seem to be a particularly suitable target for GDEPT (gene-directed enzyme prodrug therapy), in which a vector is utilised to deliver a gene which is specifically expressed in cancer cells. The gene codes for an enzyme that converts a non-toxic prodrug to a highly toxic metabolite. Delivery represents a problem that may be overcome in the case of metastatic colon cancer by hepatic intra-arterial injection.

The development of positron emission tomography (PET) as an aid to early clinical trials represents a significant advance [30]. The assessment of tumour response by measuring reduction in volume is an inaccurate and insensitive method of measuring tumour cell kill. Even in carefully controlled animal experiments, a drug which may kill greater than 90% of tumour cells may only show a small change in volume. Early clinical trials indicate that the measurement of fluorodeoxyglucose utilisation or thymidine incorporation by PET may clearly distinguish between responders and non-responders within days of drug administration. This technique should enable the rapid clinical trials of many of the approaches described above and the selection of chemicals that might otherwise not have been shown to be active.

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