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New Drug Development

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INTRODUCTION

AT THE start of the 1990s it is apparent that the evolution of chemotherapy is entering a crucial phase. After the first demonstration of the successful use of anticancer agents in human cancer over 30 years ago, there followed a period of 20 years of clinical research, largely based on empiricism, which gave rise to some remarkable successes.

The drugs which provided the basis for these achievements came from a variety of sources. Sometimes, chance observations led to the development of highly effective agents, e.g cisplatin. In other cases, specific cellular targets were identified and agents were synthesised with the aim of preventing DNA synthesis, e.g. 5-fluorouracil.

In the 1980s however, the rate of progress had slowed, and in attempting to maintain the pace of drug discovery, large scale random screening of compounds, both natural and synthetic, was set up, notably at the National Cancer Institute (NCI), USA. Unfortunately, this approach proved largely unsuccessful, and more recently a new screening method has been adopted which uses a panel of human tumour-derived cell lines and aims to identify agents with unexpected tumour specificity [1].

At the same time as drug development was entering a plateau in productivity, basic scientists in the field of cancer research were making major strides in gaining greater insight into the mechanisms which underlie both the growth and control of cancer cells. The challenge now in the 1990s for those involved in new drug discovery is to take this information forward into rational drug design. Clinicians however wish to retain a broad view of potential advances, and clearly the lessons learned and experience gained from the use of currently available agents should be utilised as part of new drug development. In broad terms, approaches to new drug development can therefore be categorised as follows.

DEVELOPMENT OF ANALOGUES OF EXISTING AGENTS

The development of analogues of existing agents has been the focus of considerable attention over the past 10 years, although recently its value has been questioned. Several agents, including cisplatin and doxorubicin, have clearly established mechanisms of actions and forms of toxicity, and certain analogues have been identified. As an alternative to cisplatin, carboplatin has emerged as a major new agent [2], but as yet no analogue has shown such a clear advantage in the case of doxorubicin. Epirubicin has shown somewhat less toxicity [3], but the real prize in analogue development of anthracyclines would be the discovery of an analogue which was clearly non-cross resistant, since doxorubicin resistance remains a major clinical problem. Studies from Twentyman's group in Cambridge point to specific molecular sites for substitution which may prove most relevant in attempting to design non-cross resistant doxorubicin analogues [4]. These include substitution at the 9-alkyl position on the A ring of doxorubicin, while the morpholinyl substitution on the daunosamine sugar seems also to be particularly important. One analogue in fact bears both these substitutions and has proved very promising in experimental tumour models. It is now in early clinical trial under the code name MX2 in Japan.

Perhaps more promising in new drug development is the effort to develop analogues of agents with intracellular sites of action whose importance has only recently been recognised. Several examples of this type of agents exist, with a range of target sites of action, and only two examples are given here, to represent drugs aimed respectively at targets within the cytoplasm and the nucleus. These are the natural product, taxol, whose target is the microtubule, and the topoisomerase I inhibitor, camptothecin.

Microtubule as target

For several years, the cytoplasmic microtubule has been recognised as an important target, since it plays an essential role during cellular mitosis as well as in cellular migration. Vinca alkaloids act by inhibiting tubulin assembly, but taxol, an agent which emerged through the process of random screening at the NCI, has the opposite action of promoting tubulin assembly. Taxol is a plant product derived from in-depth fermentation from the Western Yew tree, *Taxus brevifola*. Considerable interest has been engendered in this class of drug as a result of clinical studies which have shown it to have significant activity in patients with refractory cancer, including several with ovarian cancer progressing on previous therapy with cisplatin [5]. This activity is clearly in excess of that which would have been predicted for classical spindle poisons, and it may be that taxol represents the first example of an important new class of compound. The drug did display significant toxicity, including alopecia, vomiting and occasional allergic reactions, and its development may be limited because of a difficult extraction procedure and a finite limit to its natural source. Considerable efforts are therefore now currently underway, aimed both at identifying alternative methods of extraction of the agent, e.g. from the leaves of the tree, and also at developing synthetic analogues which will provide a more reliable drug supply [6].

Topoisomerase inhibition

Within the nucleus, some 50 cm of helical DNA undergoes replication during the process of cell division, and clearly this process requires careful control. DNA topoisomerases I and II play an essential role in this regard, and several agents are known to exert their effect by inhibiting DNA topoisomerase activity, particularly topoisomerase II. During cell division topoisomerase II, which can cleave both strands of the DNA helix, forms a protein bridge with DNA until continuity is restored; agents such as epidopodophyllotoxins stabilise this protein complex, arresting the process of strand division and allowing endogenous nucleases to degrade the DNA, causing cell death.

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Topoisomerase I is also involved in the process of breakage and resealing, but can only cleave a single DNA strand. The importance of topoisomerase I as a target for currently available agents is less clear than for topoisomerase II. Intercalating agents of the acridine group, and also actinomycin D, are known to stabilise the topoisomerase I-DNA complex, but the agent best characterised as a specific topoisomerase I inhibitor is camptothecin [7]. This agent, an alkaloid extracted from the *Camptotheca acuminata* tree in northern China, entered clinical trial in the early 1970s [8], but studies were terminated because of unpredictable toxicity such as myelosuppression, diarrhoea and stomatitis. Initial studies had reported activity in large bowel cancer, but these were not confirmed before trials were terminated.

Some recent laboratory data, however, have rekindled interest in this target as a lead for new drug design [9]. Using an immunoblot analysis for topoisomerase I, Giovenella's group showed that concentrations of topoisomerase I in surgical specimens of colon cancer, and in colon cancer xenografts, were significantly higher than in normal colonic mucosa. They next tested a series of analogues of camptothecin in three xenograft lines of colon cancer, and concluded that one compound, i.e. 20(RS)-9-aminocamptothecin (9-AC), showed the greatest promise by virtue of high levels of efficacy in all three lines (in comparison with conventional agents such as 5FU) together with appreciably less systemic toxicity than the parent compound in xenograft-bearing nude mice. This and other camptothecin analogues are at the stage of early clinical trial, the aim of which is to clarify the importance of topoisomerase I as a potentially selective target.

Other targets

Clearly there are other examples of analogue development, in which the eventual target is cellular DNA. These include new thymidylate synthetase inhibitors [10], less hepatotoxic than the parent compound CB 3717 [11], and new DNA intercalating agents, such as the anthrapyrazoles which may be less toxic than those in current use [12]. Analogues of quinolone and indole compounds (such as mitomycin) are also of interest, as they may provide a degree of selective DNA alkylation by virtue of their activation in hypoxic (tumour) cells as opposed to oxic (normal) cells [13]. However, the general view is that major strides in drug development are unlikely to come from agents whose primary target is DNA, until greater use is made of basic molecular information on potential differences between normal and malignant cells.

Newer agents are now being synthesised with high binding specificity for the minor groove of DNA, specifically for AT-rich sequences. Examples are distamycin A and derivatives of the parent drug, such as the mustard derivative FCE 24517 [14]. These agents appear to be capable of inhibiting the binding of specific nuclear proteins in the promoter region of model genes, thereby potentially preventing the recognition of regulatory transcription factors essential for the growth of cancer cells. Certain distamycin analogues are already in early clinical trials. In future other agents designed to inhibit specific sequences are likely to emerge.

NOVEL AGENTS AND "RATIONAL" DRUG DESIGN

In recent years, efforts have turned to the development of agents which are likely to have different sites of action to those in current use. As understanding increases in respect of the molecular events leading to malignant transformation, those involved in rational drug design are paying particular attention to the cell membrane and signal transduction pathways within the cell as new targets. Agents of this type may be expected to have novel structures and unusual patterns of toxicity, and they may pose particular problems for clinicians performing early clinical studies. Examples of these agents and their respective targets are given below.

The cell membrane

Functionally, the cell membrane comprises a lipid domain and growth factor receptor proteins, together with proteases and cell surface adhesion molecules surrounded by intercellular matrix proteins. Each of these presents a potential target for new drug design.

A new class of experimental agent, the ether lipids, may have an effect on the cell membrane, although individual members of this class appear to have differing mechanisms. The lead compound, ET-18-OCH_3 does increase membrane fluidity, and structurally, it is an analogue of the membrane-bound platelet-activating factor receptor. Through either or both of these properties it is demonstrated to have significant selective activity experimentally, while having no direct effect on DNA [15]. Several ether lipids are now in early clinical trial and data are awaited with interest.

Another agent whose primary target may be the binding of stimulatory growth factors to their membrane receptors is suramin, originally developed for the treatment of trypanosomiasis. It is capable of inhibiting the binding of platelet-derived growth factor (PDGF) to its receptor, as well as the binding of transforming growth factor beta (TGFβ), epidermal growth factor (EGF) and basic fibroblast growth factor (FGF). One tumour type in which growth factor stimulation, and inhibition of its receptor-binding, correlates with *in vitro* cytotoxicity is prostatic cancer [16]. As well as demonstrating efficacy *in vitro*, suramin is active in patients with refractory prostatic cancer, with initial objective response rates of the order of 40% [17]. Toxicity is significant, however, and the search is on for analogues specifically designed for their antitumour activity.

An alternative approach to the growth factor receptor as a target is the synthesis of peptide antagonists which will prevent ligand binding. One example of this is the development of antagonists of bombesin and other neuropeptides, which inhibit the growth of small cell lung cancer *in vitro* [18]. Preliminary studies in xenografts are also encouraging. Analogues of somatostatin present similar interesting potential in a range of tumours. In addition, monoclonal antibodies directed either at the receptor binding epitope of the growth factor, or the binding site of the growth factor receptor itself, present intriguing possibilities. An anti-EGF monoclonal antibody is cytostatic in tissue culture and clinical trials are underway [19].

Intracellular targets

Growth factors may stimulate receptor-mediated mitogenesis directly, if the receptor possesses intrinsic tyrosine kinase activity (e.g. EGF receptor) or indirectly via the generation of signal molecules. Often this involves an intracellular G-protein whose role is to regulate the breakdown of membrane-bound inositol lipid to the intracellular second messengers, inositol triphosphate and diacyl glycerol, which activates protein-kinase C. A number of targets thus present themselves in this cascade, and the task for the future is to identify the key enzymes in target tumour cells. Since these intracellular signals are common to both normal and tumour cells, selectivity may partly depend

on the identification of tumour specific isoenzymes as well as different levels of expression of key enzymes in tumour compared to normal cells. In this respect, inhibition of signal transduction arising from mutated *ras* p21 G-protein offers exciting possibilities for drug development. The mechanisms have now been elucidated by which the *ras* p21 G-protein undergoes structural changes (and increases in hydrophobicity) prior to membrane localisation and initiation of mitogenesis [20]. These changes offer potential for agents which inhibit metabolic steps such as cholesterol biosynthesis, although selectivity may be a limiting factor.

Considerable efforts in drug development are currently directed towards protein kinase C, focusing both on activating and inhibiting its various isoforms. An interesting group of protein kinase C agonists are the macrocyclic lactones isolated from marine bryozoars growing on the bottom of boats and known as bryostatins [21]. These are potent at low concentrations, and they may be effective by promoting membrane localisation and degradation of protein kinase C following its initial activation. *In vivo* antitumour activity has been demonstrated, and phase I trials are planned to start shortly in the UK.

Other examples of agents directed at intracellular targets involved in signal transduction include specific inhibitors of tyrosine kinase, e.g. genistein [22], while the new agent mopidamol, which acts to prevent the breakdown of the important intracellular molecule, cyclic AMP, also shows promise, with positive clinical results reported on patients with non-small cell lung cancer [23].

A further, more speculative, intracellular target which is attracting increasing interest is cytoplasmic messenger RNA. Expanding knowledge of key base sequences has permitted the development of so-called antisense oligodeoxynucleotides, their aim being to inhibit the translation of vital regulatory proteins [24]. They act either by directly cross-linking RNA, by steric inhibition, or by forming a hybrid with RNA which activates endogenous enzymes such as RNAases. Among the most interesting early studies *in vitro* are those which have demonstrated the capacity of methylphosphonate oligonucleotides specific for the *bcl-2* proto-oncogene to inhibit growth of a B cell lymphoma cell line [25]. Major problems clearly remain in considering *in vivo* application of antisense oligonucleotides; these include problems of delivery to the appropriate intracellular site, as well as problems of biological degradation. Nevertheless, their potential is considerable, not least in terms of reversing cytotoxic drug resistance e.g. by inhibiting P-glycoprotein synthesis in multi-drug resistant cancer cells with oligonucleotides specific for *mdr*-mRNA.

Tumour vasculature

Clearly there is no shortage of potential new targets for anticancer drug development, and this discussion has focused on those which present themselves at the cellular levels. However, other possibilities exist, not least of which is tumour vasculature. The pathophysiology of tumour blood flow is not fully understood, but it seems likely that some tumour cells are capable of inducing new blood vessel formation through the action of a tumour-derived polypeptide, tumour angiogenesis factor, which is specific for vascular endothelial cells [26]. Experimental data indicate that neovascularisation may be inhibited by the combination of synthetic steroids without glucocorticoid activity, e.g. cortexolone, and synthetic heparin substitutes, i.e. cyclodextrin derivatives, which are devoid of anticoagulant properties [27]. Although considerably more developmental work is required before clinical trials can be contemplated, this approach deserves further consideration, particularly as it may prove complementary to other targets.

In a similar vein, tumour vasculature appears to be a major target for the novel agent, flavone acetic acid, which attracted considerable attention recently because of its high level of experimental antitumour activity [28]. The agent proved to be inactive in phase II trials, previous phase I trials having identified hypotension as a major dose-limiting toxicity. It now seems that the drug is effective experimentally at least partly through an acute disruption of tumour blood flow, perhaps mediated indirectly by tumour necrosis factor [29], and presumably those effects contribute to its clinical toxicity. In addition, preclinical data indicate a high degree of synergy between flavone acetic acid and interleukin-2, and clinical trials of this combination are in progress. Meanwhile, it is hoped that analogue development will reveal molecules with a greater degree of selectivity, and the models for screening will require careful selection, bearing in mind the interspecies variation already observed.

CONCLUSION

Finally, it should be noted that new drug development continues to proceed alongside the development of novel forms of drug delivery. Although beyond the scope of this review, it is evident that the potential for new drug carriers, such as microspheres and polymers, and for antibody-directed therapy such as the ADEPT approach using pro-drugs and enzymes [30], is considerable. Over the next five years, it is reasonable to expect evidence of tangible benefit to cancer patients from some, at least, of these approaches.

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Management of AIDS and its Neoplastic Complications

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INTRODUCTION

THE acquired immunodeficiency syndrome (AIDS) was first reported in 1981 as a combined epidemic of *Pneumocystis carinii* pneumonia (PCP) and Kaposi's sarcoma (KS) [1–3].

This syndrome has emerged as the most important epidemic diseases infection of our time. By the middle of 1990, nearly 270 000 cases of AIDS has occurred worldwide [4]. The number of AIDS cases officially reported to the WHO represents probably one third of the real total. Delay in reporting, underreporting and inadequate means of diagnosis almost certainly contribute to this gross underestimation. The identification of the aetiological agent, human immunodeficiency virus (HIV), a human T-lymphotropic retrovirus, represents one of the most significant steps in AIDS research [5–7]. HIV may infect many human cells, including those in the brain, but is able to recognise the T4 lymphocyte surface marker and has a strong affinity for this subset of lymphocytes. The selective cytopathic effect of HIV on T4 lymphocytes results in an imbalance in the usual ratio of T4 to T8 cells, with a decline in lymphocyte recognition and response to antigen. As the response to antigen by T-lymphocytes is a prime initiator of the immune response, the lack of cellular immune response results in increased susceptibility to opportunistic infections and neoplasms, which an intact immune system will ordinarily resist. Diagnosis of AIDS was based on detection of antibodies to HIV, or other serological evidences of exposure to the virus (such as viral p24 antigen, virus production or reverse transcriptase activity), in combination with defects of cellular immunity (such as the T-lymphocyte abnormalities noted above) and the presence of characteristic opportunistic infections or neoplasms, specifically defined by the United States Centers for Disease Control (CDC). In 1987, the CDC surveillance definition for AIDS was revised to emphasise HIV infection status through the inclusion of additional indicator diseases and acceptance of presumptive diagnosis of some indicator diseases [8].

The accumulated evidence strongly suggests the conclusion that transmissison of HIV occurs only through blood, sexual activity and perinatal events.

Antiretroviral therapy of AIDS

To date, only one agent, zidovudine (also known as azidothymidine, or AZT) has been definitely shown to alter the usually rapidly fatal course of AIDS.

Zidovudine (3′-azido-3′-deoxythymidine) is a thymidine analogue that inhibits the replication of the HIV *in vitro*. It is phosphorylated by cellular enzymes to a 5′-triphosphate form that interferes with the viral RNA dependent DNA polymerase (reverse transcriptase) and chain elongation of the viral DNA,

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