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Activation of Prodrugs by Targeted Enzymes

DR SPRINGER and her associates described the use of a novel approach for tumour therapy in the current issue (p. 1361). This approach, which was introduced independently by Dr. Bagshawe's group in London [1–3] and by our group in Seattle [4–6], involves the use of monoclonal antibodies (Mabs) for the delivery of enzymes to tumour cell surfaces. The Mab–enzyme conjugates are administered systemically. After enough time is allowed for the conjugates to bind to antigens present on the tumour cells and to clear from non-target tissues, a prodrug is administered which is catalytically converted into an active antitumour agent by the targeted enzyme

There are several distinctions between such a multistep approach to solid tumour therapy and methods involving direct Mab–drug conjugates. In order for Mab–drug conjugates to be effective, several conditions must be met: (a) the Mab–drug conjugate must penetrate throughout the tumour mass and bind to the vast majority of cells; (b) the conjugate must be taken up inside the tumour cells; (c) sufficient quantities of the cytotoxic agent must be released; and (d) therapy-resistant tumour cell variants must not arise. Successful treatment can therefore be limited by poor Mab distribution, heterogeneity of antigen expression, modest drug potency and the inability of many Mabs to internalise in an appropriate fashion into cells. The use of targeted enzymes for prodrug activation is designed to overcome these limitations since a single enzyme is capable of generating large amounts of active drug. The drug is preferentially released outside the cell where it can penetrate both into tumour cells that have bound the conjugate, and into other cells within the tumour that have not. This approach has the potential of circumventing the limitations described above.

Several enzymes of both mammalian and non-mammalian origin are being explored for the activation of a wide range of prodrugs. While enzymes of mammalian origin might be advantageous due to reduced immunogenicity, the prodrugs that they act upon might be substrates for corresponding endogenous enzymes. One of the first demonstrations of the utility of the targeted enzyme-prodrug approach involved the use of Mab–alkaline phosphatase (Mab–AP) conjugates for the activation of etoposide phosphate [4, 5] and mitomycin phosphate [5]. In both cases, the targeted enzyme–prodrug combinations were therapeutically better than the prodrugs alone or the corresponding drugs produced upon hydrolysis. However, the similarities in the maximum tolerated doses for the drugs and their corresponding phosphate derivatives indicated that a substantial amount of hydrolysis was mediated by endogenous AP. By minimising the levels of undesired prodrug hydrolysis, it should be possible to increase the amount of active drug generated within the tumour.

The bacterial enzyme, carboxypeptidase G2 (CPG2), was used in the study by Dr Springer and coworkers for the activation of three related mustard prodrugs. Previous work demonstrated pronounced antitumour activity for one Mab–CPG2–prodrug combination [1]. In the work described here, parameters such as conjugate and prodrug dosages, and the schedule of prodrug administration schedule were investigated. It was found that combinations of the binding Mab–CPG2 conjugates with either of two of the prodrugs gave significant antitumour effects, providing that the conjugate and prodrug doses were high enough and that a long enough interval of time was allowed for the clearance of circulating conjugate prior to the administration of the prodrugs. The antitumour effect was most likely due to intratumoral generation of the active anticancer drug, since the non-binding conjugate did not increase the activity of one of the prodrugs. Unfortunately, no comparison was made with free drug in this paper. Although a previous publication [1] demonstrated the drug to have little antitumour activity, this comparison should be made on a routine basis in order to establish the relative merit of this approach compared to conventional cytotoxic chemotherapy.

Other enzymes that are currently being investigated for the activation of prodrugs include penicillin amidase [7], β–lactamase [8, 9], cytosine deaminase (P.D.S. *et al.*), and nitroreductase [10]. In choosing an enzyme that has the greatest probability for success, it will be necessary to consider its physical properties and its stability under physiological conditions, as well as the nature of the drug that the enzyme releases. In addition, the rate of conjugate clearance and its uptake and distribution within the tumour will depend on which enzyme is used, and undoubtedly will influence the therapeutic outcome. Yet another variable concerns the kinetics of drug release by the targeted enzyme. It would be of interest to test the validity of recent pharmacokinetic model studies suggesting that low turnover rates may result in high tumour to blood drug ratios if the conjugate concentration in the tumour is higher than in the blood [11]. These same analyses also suggest that under some conditions there may be advantages in enzymes with high K_m values.

A vast array of drugs can be generated using Mab–enzyme conjugates. Of the many possibilities, there are advantages in releasing drugs whose clinical activities are already well understood, since the therapeutic results and regulatory issues are likely to be more predictable. Further advantages may be gained by selecting enzymes with broad substrate specificities, because of the possibility of using a single Mab–enzyme conjugate with prodrug combinations. Experiments along these lines have been reported for alkaline phosphatase with two mechanistically unrelated prodrugs [4, 5], and for CPG2 with a series of alkylating agents by Springer *et al.*

The activation of prodrugs by targeted enzymes is a new field with encouraging potential. In a relatively short period of time, several laboratories have demonstrated interesting and significant activities using this approach, and clinical studies have already begun [12]. Detailed pharmacokinetics studies and elucidation of the factors influencing therapeutic efficacy in model systems should provide mechanistic insight and a basis for developing useful clinical reagents.

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Anal Intraepithelial Neoplasia

ANAL INTRAEPITHELIAL NEOPLASIA (AIN) may be defined as the presence of nuclear abnormalities in the anal epithelium in the absence of inflammation without breach of the basement membrane. It was first described by Fenger and Nielsen [1] who reviewed all the anal surgical pathology specimens in the whole of Denmark over a 2-year period [2] and found 19 cases of AIN and graded them according to the cervical intraepithelial neoplasia (CIN) system. In AIN 1, nuclear abnormalities are restricted to the lower third of the epithelium, in AIN II the lower two-thirds are affected, and in AIN III nuclear abnormalities are present throughout the full thickness of the epithelium.

The most frequent site of AIN would appear to be the anal transitional zone. This is an area of transitional epithelium that lies between columnar epithelium above and squamous epithelium below, similarly to the cervical transitional zone. AIN lesions have also been identified in the squamous epithelium of the perianal skin and in certain patients both sites may be affected.

The prevalence of AIN, its clinical significance, whether it is analogous to CIN where a proportion may progress to invasive cancer and whether lesions associated with certain human papillomavirus (HPV) types have a more aggressive course remains unknown.

Although its exact role is unclear, HPV seems to be associated with cervical intraepithelial neoplasia, with certain types, notably 16 and 18, appearing to be associated with more severe grades of CIN and invasive carcinoma of the cervix [3]. HPV type 16 is increasingly frequently being identified in squamous cell carcinoma of the anus [4] and has also been identified in AIN lesions [5]. Although the number of patients with AIN in most reports is small, AIN III lesions do appear to contain HPV 16 more frequently than lesser grades of AIN [6].

Fenger and Nielsen [2] originally felt that AIN was a rare condition; however subsequently several groups have been identified in whom there is a high prevalence of AIN, in particular homosexual men with anal condylomata and to a lesser extent heterosexual men and women with anogenital warts [5–7].

Homosexual men commonly develop anal condylomata and appear to be at an increased risk of developing anal cancer, a malignancy which is becoming increasingly prevalent in this group [8,9]. Studies from the USA have demonstrated a high prevalence of AIN in HIV-positive homosexual and bisexual patients, particularly in those with advanced HIV infection and anal condylomata [10,11]. An examination of cancer registration in New York looked at the incidence of various types of cancer in men deemed to be at risk of AIDS and compared the period 1977–1985 with a pre-AIDS period of 1973–1976 [12]. Predictably, the greatest increase was in the incidence of Kaposi's sarcoma followed by non-Hodgkin lymphoma; however, the only other cancer that showed a significant increase was anal cancer. At present Kaposi's sarcoma and non-Hodgkin lymphoma are the only two tumours recognised as being definitely associated with HIV. However, it is possible that as our ability to look after HIV-positive patients improves and these patients continue to live longer in an immunosuppressed state, other tumours including anal cancer may become more common. Should that be the case, the identification of possible precursor lesions in the form of AIN may be increasingly important.

The association between anal cancer and immunosuppression was well established before the recognition of the HIV epidemic. Studies primarily of renal transplant recipients have demonstrated that immunosuppressed patients are at an increased risk of developing several malignancies including cancer of the

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