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Targeted delivery of chemotherapeutics: tumor-activated prodrug therapy

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Abstract

The potential of targeted delivery of chemotherapeutic drugs for the treatment of cancer has not yet been realized owing to the difficulty of delivering therapeutic concentrations to the target site. While in vivo studies in animal tumor models have produced very encouraging results, clinical studies with antibody–drug conjugates have been less successful. This paper will review the current status of the targeted delivery approach and analyze some of the reasons for the lack of success so far. Starting with a historical perspective, this review will end with a description of newer, more potent and specific antibody–drug conjugates, which behave like tumor-activated prodrugs that may yet fulfil the promise of the targeted delivery approach for the treatment of cancer. © 1998 Elsevier Science B.V.

Keywords: Antibody–drug conjugates; Cancer; Prodrugs; Anti-cancer agents

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1. Introduction

Cancer chemotherapy today relies on the expectation that anti-cancer drugs will preferentially kill rapidly proliferating tumor cells rather than normal cells. Typically, cancer patients with disseminated disease present themselves with approximately 10^{12} tumor cells, and it is well established that at least 99% of these cells have to be killed (i.e. a two-log or

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greater cell kill) to achieve a complete remission. Continued treatment during remission is required to achieve complete eradication of the tumor. A schematic representation of the drug treatment categories for cancer as originally described by Frei [1] is shown in Fig. 1. Unfortunately, clinically used anti-cancer drugs have limited selectivity for the tumor. The levels of drug required to kill sufficient number of tumor cells to achieve and maintain a state of complete remission in patients causes significant toxicity towards actively proliferating non-malignant cells, such as normal cells of the gastrointestinal tract and bone marrow. Thus, a continuing challenge in cancer treatment is to develop new cytotoxic agents with greater selectivity for the tumor. To achieve this goal, it is first necessary to identify inherent differences between normal and cancer cells that can be potentially exploited.

The discovery that tumor cells expressed specific determinants on their cell surface that were not found on normal cells suggested that this distinction could form the basis for the selective targeting of tumors. The advent of monoclonal antibody technology [2] led to the development of a myriad of monoclonal antibodies, each with its own binding specificity for novel tumor-specific antigens. The logical outcome was to exploit the binding specificity of the antibody to deliver a cytotoxic agent selectively to the tumor site with the hope of delivering a high, lethal concentration of drug to the target cells. The cytotoxic agent could be in the form of a protein toxin, a radioisotope or a small cytotoxic drug. This chapter will review the progress made in the area of targeted delivery of small chemotherapeutic drugs, discuss some of the shortcomings of the earlier approaches, and provide potential solutions that may help restore the promise of the targeted delivery approach for cancer therapy.

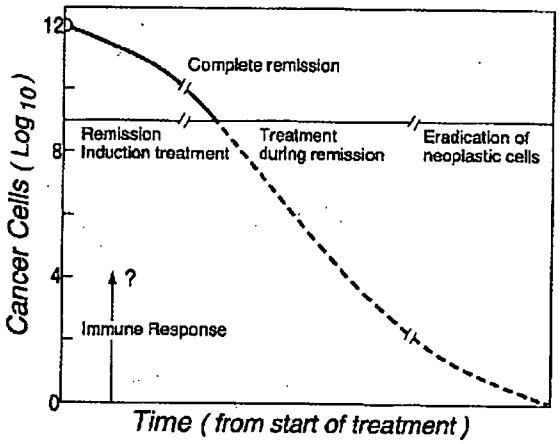

Fig. 1. Chemotherapy of cancer (reproduced with permission from Frei [1]).

2. Tumor-activated prodrug (TAP) therapy

The basic premise of the targeted delivery approach is that conjugation of drug to a tumor-specific molecule renders the drug inactive until it reaches the target site. Once at the tumor site, the conjugated drug binds to the surface of tumor cells and is further processed (internalized, released from the carrier molecule) to restore its original potency. Thus, drug conjugates can be considered as tumor-activated prodrugs (TAPs). While most conventional prodrugs are converted to active drugs by mechanisms such as chemical or enzymatic hydrolysis, restoring the activity of TAPs should ideally be dependent on interaction with antigens or receptors specifically found on the surface of tumor cells. Historically, conventional prodrugs have been designed to overcome a physiological barrier, such as poor oral bioavailability or rapid metabolism [3,4]. Often, the oral delivery of a drug is improved by merely converting it into a water soluble prodrug. Conventional prodrugs are designed with the expectation that improving the pharmacokinetic properties of a parent drug will result in increased levels in circulation and thus greater levels at the target site. In TAP therapy, the specific affinity of the tumor-associated antigen or receptor for the targeting component of the drug conjugate results, in addition, in a greater uptake and retention of the TAP at the targeted tumor site and, therefore, in increased selectivity. This is followed by liberation of the active drug resulting in high local concentration at the target site. Ideally, TAPs would be stable during circulation such that no conversion of the prodrug to the active form occurs outside the targeted tumor. Also, TAPs would not bind to non-target tissues and thus will be non-toxic while in circulation in vivo.

The principle of drug conjugates as tumor-activated prodrugs is illustrated with antibody–drug conjugates as an example in Fig. 2. In an ideal

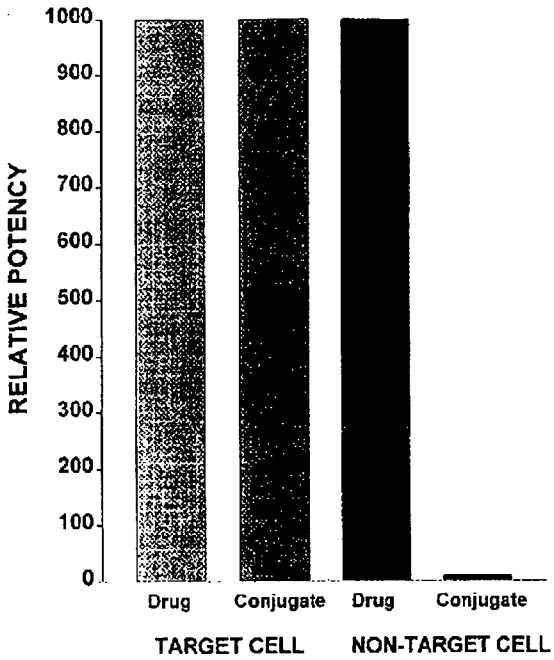

Fig. 2. Relative potency of free drug and antibody–drug conjugates (tumor-activated prodrugs, TAPs) towards target and non-target cells.

situation, for an antigen-negative cell which does not bind conjugate, conversion of the free drug into a TAP by conjugation to an antibody results in inactivation of the drug. For an antigen-positive cell, binding to the TAP is followed by internalization and release of the free drug in its fully active form. Thus the free drug and TAP have equal potency for the target cell. The therapeutic window is determined by the difference in cytotoxicity of the TAP for the target versus the non-target cell. As we will see later, the effectiveness of TAP therapy depends on several factors including choice of the targeting molecule, the potency of the drug and the nature of the release mechanism for conversion of the prodrug into the active drug.

3. Tumor-specific agents

The success of the targeted drug delivery approach for the treatment of cancer relies to a great extent on the tumor-specificity of the targeting agent. The cell surface molecule which the targeting agent recognizes can be a tumor-specific antigen (typically a glycoprotein, carbohydrate or oncoprotein), a growth factor receptor, or a receptor for a hormone. Ideally, the cell surface molecule would have the following properties: (a) well defined molecule expressed exclusively on tumor tissue, (b) not expressed on normal tissues (c) binds to the targeting molecule with high affinity, (d) expressed homogeneously on all target tumor cells, (e) present on the tumors of all patients with the same type of cancer (f) not shed into the serum of patients.

In the early phase of the targeted therapy approach, monoclonal antibodies were heralded as ideal targeting agents that bound exclusively to antigens expressed on tumor cells. However, the use of more sensitive analytical techniques such as immunofluorescence and immunohistochemical staining revealed that most antibodies bound to tumor-associated antigens that were only preferentially expressed on the surface of tumor cells. In most cases, the antibodies also bound to varying extent to antigens found on a limited number of normal tissues. In fact, the target antigens for most antibodies [5,6] developed against solid tumors were selected mainly on the basis of the higher expression of the antigen on tumors in comparison with normal tissues. The only truly tumor-specific antigens appear to be those found in hematopoietic tumors, such as idiotypes present on the surface of B-cell tumors [7], and the T-cell receptor expressed in T-cell leukemia and lymphoma. Although the cross-reactivity of antibodies with normal tissues is a matter of concern, the benefit potentially gained from the improvement in the therapeutic window of cytotoxic drugs by conjugation to antibodies often outweigh the toxicity concerns. Of course, selection of antibodies with an acceptable cross-reactivity profile is important. In addition, thorough pre-clinical toxicology studies in animals that bear the same antigenic determinants and show similar cross-reactivity patterns to that found with human tissues is critical.

Monoclonal antibodies are also attractive as targeting agents because of their high binding affinity for their respective antigens. This should allow for the localization and retention of high concentrations of drug at the tumor site. In addition, the long circulation time of antibodies also allows for a greater probability that the drug will reach the tumor site.

The therapeutic potential of conjugates of cytotoxic drugs with monoclonal antibodies derived from murine hybridomas is dampened by the development of a predictable anti-globulin immune response in humans. The generation of a human anti-mouse antibody (HAMA) response leads to rapid neutralization and clearance of the immunoconjugate from the blood stream, thus limiting its therapeutic utility. Recent advances in recombinant DNA technology, and knowledge of antibody gene structure have been applied to the engineering of rodent antibodies to make them less immunogenic. A 'humanized' antibody is constructed by transferring the murine complementarity determining regions (CDRs) on to an appropriate human framework region. Since CDRs form the antigen combining site, a humanized or CDR-grafted antibody preserves the murine antigen specificity, but because most of the antibody structure is human, it is likely to be less immunogenic in patients than the parent mouse antibody. Recent clinical studies [8] with humanized antibodies in 46 patients have demonstrated that unlike human-mouse chimeric antibodies, CDR-grafted antibodies were found not to induce a primary immune response, even after several courses of treatment. However, humanization by CDR-grafting often results in an antibody with a lower binding affinity than the parent murine antibody. Further amino acid substitutions in the framework region are usually required to maintain the correct conformation of the CDRs. Even with this improvement, CDR-grafted antibodies with somewhat lower affinity than the parent antibodies are often produced. A newer technique called variable domain resurfacing [9] takes advantage of the generally accepted view that the antigenicity of proteins is determined solely by surface epitopes. In this approach, the binding affinity is maintained by retaining the CDRs and the core of the murine variable region framework. Only the surface residues in the murine variable region are replaced by those from a human variable region. This technique was applied to two murine antibodies and, in both cases, affinity was fully preserved [10].

Although monoclonal antibodies have been the most commonly used targeting agent for chemotherapeutics, the pharmacodynamics of these large immunoglobulin molecules may impede their ability to access or penetrate solid tumors which are often poorly vascularized. The use of smaller antibody fragments instead of whole IgG molecules may be advantageous in some cases. Three comparative studies [11–13] of the tumor localization of radiolabeled intact IgGs and smaller fragments [Fv, Fab', $F(ab')_2$] in mice have shown that smaller fragments penetrate the tumor faster (maximum tumor penetration of Fv is at 0.5 h) than intact IgG, which showed an equivalent degree of tumor penetration only at 48 h post-injection. However, the smaller fragments displayed faster clearance and delivered lower overall tumor doses than the intact IgG, suggesting that conjugates with intact IgG molecules may be preferable for the specific application.

Although monoclonal antibodies probably provide the greatest binding selectivity for cancer cells, other targeting agents that preferentially bind to tumor cell surface markers may provide distinct advantages such as smaller size, rapid internalization and non-immunogenicity. For example, the epidermal growth factor receptor (EGFR) gene is amplified in a high proportion of human squamous carcinoma cell lines [14]. Levels of EGFR are three to sixty-four-fold higher in several tumor types, such as lung, breast and head and neck, as compared to that found on normal keratinocytes [15]. Human EGF is a single polypeptide of 53 amino acids and is specially attractive as a targeting agent because of its small size ($M_r = 6201$), high binding affinity for its receptor (apparent $K_d = 2–4 \times 10^{-10}$ M) and its rapid internalization upon binding to the receptor [16]. In addition human EGF will not be immunogenic. An example of another polypeptide that can also be used to target EGFR-expressing carcinomas is transforming growth factor alpha (TGFα) [17]. Other examples of low molecular weight targeting agents that appear to bind preferentially to tumors include melanocyte stimulating hormone (MSH) against melanomas [18], thyroid stimulating hormone (TSH) and thyrotropin against thyroid cancers [19], and interleukin 2 (IL2) for T-cell leukemias [20].

4. Early antibody–drug conjugates

Early antibody–drug conjugates were comprised of a monoclonal antibody covalently linked to several molecules of a clinically used anti-cancer drug.

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