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Prodrug-based intracellular delivery of anticancer agents

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SUMMARY

There are numerous anticancer agents based on a prodrug approach. However, no attempt has been made to review the ample available literature with a specific focus on the altered cell uptake pathways enabled by the conjugation and on the intracellular drug-release mechanisms. This article focuses on the cellular interactions of a broad selection of parenterally administered anticancer prodrugs based on synthetic polymers, proteins or lipids. The report also aims to highlight the prodrug design issues, which are key points to obtain an efficient intracellular drug delivery. The chemical basis of these molecular concepts is put into perspective with the uptake and intracellular activation mechanisms, the *in vitro* and *in vivo* proofs of concepts and the clinical results. Several active targeting strategies and stimuli-responsive architectures are discussed throughout the article.

Contents

Abbreviations: 5-FdU, 5-fluoro-2′-deoxyuridine; Ag, Antigen; ApoE, Apolipolipoprotein E; AraC, 1-β-D-Arabinofuranosylcytosine (cytarabine); BE, Benzyl elimination; CALI, NAc-gamma calicheamycin; CPP, Cell-penetrating peptide; CPT, Camptothecin; DAUN, Daunorubicin; dFdC, 2′,2′-difluorodeoxycytidine (gemcitabine); DOX, Doxorubicin; DMPC, Dimyristoylphosphatidylcholine; DSC, Differential scanning calorimetry; EPR, Enhanced permeability and retention; FA, Folic acid; FA-R, Folic acid receptor; FITC, Fluorescein isothiocyanate; HA, Hyaluronic acid; HPMA, *N*-(2-hydroxyhropyl)methacrylamide; LDL, Low-density lipoprotein; LDL-R, LDL Receptor; LHRH, Luteinizing hormone-releasing hormone; MAb, Monoclonal antibody; MDR, Multidrug resistance; MMAE, Monomethyl auristatine E; MMC, Mitomycin C; MTD, Maximum tolerated dose; MTX, Methotrexate; Mw, Molecular weight; NOAC, N4-octadecyl-AraC; NSCLC, Non-small-cell lung cancer; PAA, poly(aspartic acid); PABC, Para-aminobenzyl carbamate; PAMAM, Poly(amidoamine); PCT, Paclitaxel; PEG, Poly(ethylene glycol); P-gp, P-glycoprotein; PHEG, Poly-[N5-(2-hydroxyethyl)-L-glutamine]; PLA_2, Phospholipase A_2; RME, Receptor-mediated endocytosis; SMVT, Sodium-dependent multi-vitamin transporter; SQ, Squalene; SST, Somatostatin; TML, Trimethyl lock lactonisation.

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Cancer is a multifaceted disease that represents one of the leading causes of mortality in developed countries. Due to the societal and economical implications of this pathology, tremendous efforts have been made over the past decades to improve the available therapeutic options. Although a large number of potent chemotherapeutic anticancer agents have been identified and successfully used in clinical practice, considerable research activity is devoted to discover more potent treatments, while minimising their toxic side effects. Indeed, most anticancer agents display a narrow therapeutic window due to their lack of selectivity against cancer cells. Besides, the ability of the anticancer compounds to actually reach their target is often impaired by a number of physiological barriers (i.e., tumour interstitial pressure, diffusion through the tumour endothelium and/or extracellular matrix and so on) as well as by metabolisation/degradation phenomena such as conversion into inactive metabolites.

Due to the constant progress accomplished in the fields of chemistry, soft-matter science and nanotechnology and in the understanding of the biological mechanisms of cancer diseases, several drug delivery approaches have been developed to enhance the efficacy of existing anticancer agents. One of them, the 'pro-drug' strategy, was devised 50 years ago to help drugs to cross physiological barriers. The concept consists in grafting a molecule (termed 'promoiety') onto an active drug molecule that will help it in reaching the pharmacological target, while ensuring that the promoiety can afterwards be removed to regenerate the biologically active compound [1]. A well-thought-out prodrug strategy may overcome various obstacles such as poor drug solubility, the systemic conversion into inactive metabolites, a lack of site specificity or an inefficient cell uptake. This last point is especially critical in the case of anticancer therapy, as an alteration of drug transport across the cell membrane is a common mechanism of resistance to chemotherapy.

Many prodrugs described in the literature have a different cell uptake pathway than their parent drug and may ensure an efficient intracellular drug release. However, no attempt has been made to review this ample literature with a specific focus on these altered cell uptake pathways and on the intracellular drug-release mechanisms. Thus, the aim of this report is to discuss the relevant cellular mechanisms and to identify the critical design features that lead to an efficient intracellular delivery of the active molecule when administered as a prodrug. We aimed to cover the broadest possible selection of prodrugs based on synthetic polymers, lipids or proteins, and coupled to small-molecular-weight cytotoxic anticancer agents. The scope has been restricted to parenterally administered systems because prodrugs designed for non-parenteral routes usually aim to address the absorption of the compound rather than its cellular uptake in the target tissue. We have also excluded from this report several clinically important anticancer prodrugs (e.g., cyclophosphamide) [2] and two-step prodrug strategies such as enzyme- and andibody-directed enzyme prodrug therapy, which have been reviewed elsewhere [3,4] and did not fit our focus on conjugated small-molecular-weight anticancer agents. Given the magnitude of the available literature, only reports of drug-delivery strategies that provided mechanistic insight or illustrated the critical design issues were selected, at the expense of exhaustiveness.

This article is organised as follows. Each of the first two sections will describe one of the available uptake pathways for anticancer prodrugs: (1) the endocytosis of macromolecular and/or targeted constructs and (2) the passive diffusion through the plasma membrane enabled by a hydrophobic promoiety. Successful technical options to trigger the intracellular release of the active drug will be discussed in a third section. The desirable design features identified throughout the report will be summarised and put into perspective in the last section.

1. Prodrug endocytosis

Endocytosis refers to the deformation or invagination of a cell's plasma membrane, which results in the internalisation of solutes or material bound to the cell membrane or present in its vicinity. This generic concept encompasses several distinct mechanisms, such as phagocytosis, clathrin-mediated endocytosis, caveolar endocytosis or macropinocytosis [5]. The complex molecular basis and physiological relevance of the respective pathways have been reviewed in depth by several authors [6–8], and will not be detailed here. Endocytotic pathways have been used for over 30 years to deliver various payloads inside cells by means of nanometre-scale carriers [5]. In this first section of the article, we will report prodrug architectures that result in an uptake through an endocytotic pathway.

To facilitate the presentation of the prodrug architectures reported throughout the article, the chemical formulae of all anticancer agents will be replaced by their cartoon representation, to better focus on the chemical moieties relevant to the conjugation processes. The complete formulae of the drugs and their graphical placeholders are reported in Fig. 1.

1.1. Fluid-phase endocytosis

Over the past decades, different types of hydrophilic polymers have been covalently linked to various anticancer agents to improve their solubility or to alter their transport properties [9]. However, the important size and hydrophilicity of those conjugates prohibit their spontaneous diffusion through cellular membranes. Nonetheless, the fluid-phase endocytosis of the drug–polymer conjugates present in the vicinity of the cells through any of the endocytotic mechanisms mentioned above constitutes a valid cell uptake mechanism. 'Fluid-phase endocytosis' is a generic term that designates the internalisation of materials present near the cell surface (or adsorbed onto it) over the course of physiological or baseline endocytotic processes (Scheme 1). Noteworthy, to maximise the impact of this aspecific endocytosis pathway, it is essential to maximise the prodrug concentration within the tumour tissue, in the vicinity of the target cells.

1.1.1. Passive targeting: the EPR effect

Fortunately, colloidal systems such as polymer–drug conjugates display the interesting property of allowing the passive targeting of solid tumours. Indeed, the architecture of the abundant neovasculature required for tumour growth is known to be incomplete. This results in a superior permeability with respect to healthy vessels,