Journal of Controlled Release 205 (2015) 58–69

Contents lists available at ScienceDirect

Journal of Controlled Release

journal homepage: www.elsevier.com/locate/jconrel

15 years of ATTEMPTS: A macromolecular drug delivery system based on the CPP-mediated intracellular drug delivery and antibody targeting

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ARTICLE INFO

Article history:
Received 30 September 2014
Received in revised form 18 November 2014
Accepted 1 December 2014
Available online 4 December 2014

Keywords:
ATTEMPTS
CPP-mediated intracellular drug delivery
Antibody targeting
Prodrug

ABSTRACT

Traditionally, any drug intended for combating the tumor would distribute profoundly to other organs and tissues as lack of targeting specificity, thus resulting in limited therapeutic effects toward the tumor but severe drug-induced toxic side effects. To prevail over this obstacle of drug-induced systemic toxicity, a novel approach termed "ATTEMPTS" (antibody targeted triggered electrically modified prodrug type strategy) was designed, which directly introduces both of the targeting and prodrug features onto the protein drugs. The ATTEMPTS system is composed of the antibody targeting component consisting of antibodies linked with heparin, and the cell penetrating peptide (CPP) modified drug component. The two components mentioned above self-assembled into a tight complex via the charge to charge interaction between the anionic heparin and cationic CPP. Once accumulated at the targeting site, the CPP modified drug is released from the blockage by a second triggering agent, while remaining inactive in the circulation during tumor targeting thus aborting its effect on normal tissues. We utilized the heparin-induced inhibition on the cell-penetrating activity of CPP to create the prodrug feature, and subsequently the protamine-induced reversal of heparin inhibition to resume cell transduction of the protein drug via the CPP function. Our approach is the first known system to overcome this selectivity issue, enabling CPP-mediated cellular drug delivery to be practically applicable clinically. In this review, we thoroughly discussed the historical and novel progress of the "ATTEMPTS" system.

1. Introduction

Current anti-cancer drug therapies utilizing the traditional small molecular agents would result in negative therapeutic efficacy by three hurdles: lack of selectivity of cancer cells over normal cells, which would introduce the toxic side effect toward normal tissues [1,2]; the second limitation lies in the rapid clearance of these water-soluble small molecular agents from the bloodstream and the last but the largest obstacle is the drug resistance commonly exhibited by tumor cells, which leads to the reduced drug accumulation [3–6]. Therefore, the traditional anti-cancer agents tend to localize in normal tissues rather than in tumors, thereby rendering higher toxic effects.

Macromolecular agents, like protein, antibody, enzymes and nucleic acid drugs, possess several attributes such as high specificity, high solubility, optimum activity under physiological conditions, and a repetitive reaction mechanism over small molecular agents [7]. However, despite these superior efficiency, the success oncological application of these agents is beset by several limitations, such as the proteolytic degradation property, the inability of these macromolecular agents to cross cell membranes and immunogenicity. Thus, only few of these macromolecular agents have currently been approved for clinical use [1,7,8]. In the past 10–20 years, approaches have been developed to enhance the anti-cancer efficiency with fewer side effects based on the constant progress accomplished in modern science and nanotechnology [9–14]. Among all these approaches, the molecular target drug delivery method and the prodrug strategy drew much attention. The target drug delivery became prevalent, attributing to the least toxic but promising therapeutic effects. This tendency, somewhat fashionable, is now rather problematic caused by the great genetic diversification during the process of genetic alteration in human cancer [15].

Prodrug was consisted of the grafting group and the active drug, which forms an inactive formulation. In general, a well-thought-out

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http://dx.doi.org/10.1016/j.jconrel.2014.12.002
0168-3659/

prodrug strategy could achieve tissue-specific actions and reduce the undesired toxic effects, as the prodrug was designed according to the difference between the target environment and the abnormal physicochemical properties, like pH, temperature, over-expressed enzymes, receptors, and transporters etc. Considering that the common mechanism of the resistance to chemotherapy is the inability of drug transport across the cell membranes, transport moiety is especially critical in the case of anticancer therapy for protein drug [16], and various prodrug strategies were developed to achieve the selectivity issue [16–21].

Except for Prodrug strategy, various approaches based on carriers, like antibodies [22], tumor homing peptides [23–25], various nanoparticles [26–31], red blood cells [31–33], or even small molecule ligands [34,35] have been utilized in the purpose of directing the macromolecular drugs only to the cancer cells [36,37]. Our strategy, therefore, combines both of the attributes of prodrug and target drug delivery methods into a single delivery system, expecting to deliver the macromolecular drugs to specific tissue targets with minimal toxicity. Once accumulating at the site of target tissue, the prodrug would be converted to its active form at the target site, for example the tumor tissue, while keeping inactive during targeting. One of the famous applications termed "ADEPT" (Antibody Directed Enzyme Prodrug Therapy) based on a specific enzymatic conversion of the prodrug into active form at the target tissue was designed abiding by the similar principle with us [1]. However, the ADEPT system is designed toward small and hydrophobic drugs (e.g. doxorubicin), which is not suitable for macromolecular agents like protein etc. Protein drugs, for example enzymes, possess multiple functional groups making it difficult to create a single prodrug from an enzyme while keeping its activity; hence, developing an effective delivery system for macromolecular agents is both an imperative and beckoning endeavor [7].

We developed another approach termed "ATTEMPTS"(Antibody Targeted Triggered Electrically Modified Prodrug Type Strategy) [1,2,7,38–40], which directly introduces the prodrug feature onto the protein drugs, like t-PA and toxin, while after the accumulation at the targeting site, the drug is released from the blockage by a second triggering agent [41]. This system is based on the reversible masking/demasking between the cationic cell penetrating peptide (CPP) and the anionic heparin, where the clinically approved cationic protamine is used as the trigger agent [42–44]. As known, CPPs tend to bind to the anionic constituents of the cell surface, e.g. phospholipids and glycosaminoglycans, mediating delivery for the macromolecular drugs without any selection. A simple yet effective way to control the "Trojan horse" character of the CPPs is reversibly masking the cationic CPPs with anionic materials e.g. heparin and hyaluronic acid [36,37,45]. In our designed ATTEMPTS system, binding the anionic heparin to CPP would offer critical advantages: (1) it would inhibit the trans-membrane activity of CPP during the targeting process, thereby prohibiting the complex from entering normal cells; (2) without positive charge the CPP modified drug can keep away from the degradation by plasma trypsin-like proteases in circulation; and (3) it would offer a better targeting function of the antibody as the CPP is completely masked. The in vitro and in vivo feasibility of this strategy was proved in our studies. In this article, we thoroughly summarize the development of this ATTEMPTS system.

2. The design and development of the ATTEMPTS

2.1. The concept of ATTEMPTS

The CPPs modified "ATTEMPTS" system as illustrated in Fig. 1, this system is composed of two components: (i) the anionic targeting part: an antibody-heparin conjugate, (ii) the anionic effector component: the CPP modified drug, and the two components formed a plasma-stable tight complex between the targeting component and the effecter component by a charge–charge interaction. Once the

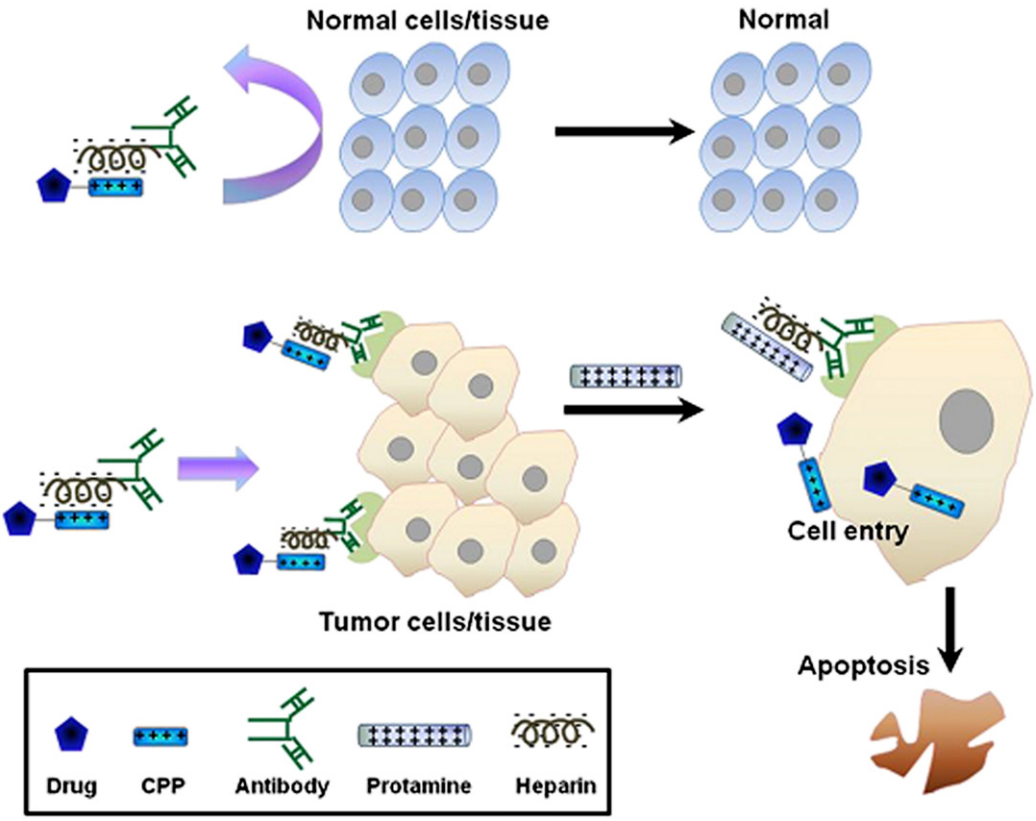

Fig. 1. Illustration of CPP-modified ATTEMPTS system [36].