Bioorganic & Medicinal Chemistry Letters 28 (2018) 3015–3024

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Digest

Effective cancer therapy based on selective drug delivery into cells across their membrane using receptor-mediated endocytosis

Toshihiko Tashima

Tashima Laboratories of Arts and Sciences, 1239-5 Toriyama-cho, Kohoku-ku, Yokohama, Kanagawa 222-0035, Japan

ARTICLE INFO

Keywords:
Receptor-mediated endocytosis
Drug delivery
Membrane permeation
Antibody drug conjugate
Tumor homing peptide
Cell penetrating protein
Cancer
Cancer therapy

ABSTRACT

Cancer is one of the major causes of death globally. The current treatment options are insufficient, leading to unmet medical needs in cancer treatment. Off-target side effects, multidrug resistance, selective distribution to cancerous tissues, and cell membrane permeation of anti-cancer agents are critical problems to overcome. There is a method to solve these problems by using receptor-mediated endocytosis (RME). It is well known that proteins such as integrin, HER2, EGFR, or other cancer biomarkers are specifically overexpressed on the surface of target cancer cells. By taking advantage of such specific receptors, payloads can be transported into cells through endocytosis using a conjugate composed of the corresponding ligands connected to the payloads by an appropriate linker. After RME, the payloads released by endosomal escape into the cytoplasm can exhibit the cytotoxic activity against cancer cells. Cell-penetrating peptides (CPPs), tumor-homing peptides (THPs), and monoclonal antibodies (mAbs) are utilized as ligands in this system. Antibody drug conjugates (ADCs) based on RME have already been used to cure cancer. In addition to the canonical conjugate method, nanocarriers for spontaneous accumulation in cancer tissue due to enhanced permeability and retention (EPR) effect are extensively used. In this review, I introduce the possibilities and advantages of drug design and development based on RME for the treatment of cancer.

Introduction

In drug discovery and development, cell permeability of drugs is a serious obstacle to directing their activity inside the cells that are targeted for curing diseases. Drugs are subject to strong pharmacokinetic profiling, although there are several medication methods, including administration by oral, intravenous, and other routes. Actually, many drug candidates have been discarded during clinical trial studies due to pharmacokinetic problems rather than pharmacodynamic problems. Bioavailability is the key issue with these drugs. Therefore, improving drug absorption, distribution, metabolism, excretion, and toxicity (ADMET) is essential to drug discovery and development. Medicinal chemists and pharmaceutical scientists must overcome this obstacle.[1] Thus, drug design is very important in order to move the impermeable active compounds into cells across the membrane. Moreover, developing effective cancer therapy to fulfill unmet medical needs is a challenging proposition because the off-target side effects and anti-cancer drug resistance in present cancer therapy are serious problems. The establishment of selective drug delivery into cancer cells is greatly needed.

There are some types of drugs that are divided into low-molecular compound (MW < ca.500), high-molecular compound (MW > ca.3000), and middle-molecular compound (MW ca.500–ca.3000) categories. Low-molecular compounds, which include typical pharmaceutical agents following Lipinski's rule of 5,[2] cross the membrane not only by passive transport based on passive diffusion, but also by active transport based on transporters. However, MDR1 (P-gp), a representative ABC-transporter, is expressed at the BBB and in cancer cells. As a result, drugs are effluxed by MDR1s in the cell membranes, which causes resistance to anti-cancer agents in cancer cells. To overcome this phenomenon, transporter-conscious drug design[3] has attracted attention. Theoretically, well-designed substrates of arbitrary

Abbreviations: MW, molecular weight; MDR1, multiple drug resistance 1; P-gp, P-glycoprotein; ABC-transporter, ATP-binding cassette transporter; ATP, adenosine triphosphate; BBB, blood brain barrier; SLC transporter, solute carrier transporter; logP, partition coefficient; RNA, ribonucleic acid; H^+, proton; ATPase, adenosine 5′-triphosphatase; pH, potential of hydrogen; EIPA, 5-(*N*-ethyl-*N*-isopropyl)amiloride; Arg, arginine; HER2, human epidermal growth factor receptor type2; a.a, amino acid; EGFR, epidermal growth factor receptor; CD, cluster of differentiation; PEG, polyethylene glycol; Lys, lysine, GFP, green fluorescent protein; Gly, glycine; Asn, asparagine; FITC, fluorescein isothiocyanate; DSPE, distearoyl phosphatidyl ethanolamine; ADC, antibody-drug conjugate; uPARAP, urokinase plasminogen activator receptor-associated protein

E-mail address: tashima_lab@yahoo.co.jp.

https://doi.org/10.1016/j.bmcl.2018.07.012
Received 11 April 2018; Received in revised form 28 June 2018; Accepted 4 July 2018
Available online 11 July 2018
0960-894X/

SLC transporters expressed near MDR1s will enter into cells. It is known that SLC transporters display tissue-specificity. Thus, designing low-molecular drugs is a useful tactic for effective drug delivery. As molecular size increases, delivery into intended cells becomes more challenging. It is true that some middle-molecular compounds are transported into cells by transporters, but pore diameter of transporters is restrictive. Moreover, some cyclic peptides or *N*-alkylated peptides are internalized through passive diffusion. Therefore, it is difficult for even middle-molecular compounds to penetrate the cell membranes due to molecular size and logP. Even worse, high-molecular compounds cannot penetrate the cell membrane in normal cases. However, well-designed and well-modified compounds can be transported into cells across the membrane regardless of their molecular size.

Recently, it was revealed that exosomes containing certain substances such as proteins and non-coding RNAs are expelled from cell membranes to other recipient cells during cell-cell communication. It is expected that substance delivery into cells can be established using endosomes. Intriguingly, viruses deliver their nucleic acids and proteins into the host cells across the plasma membrane using cell penetrating proteins (CPPs), which induce receptor-mediated endocytosis (RME). Therefore, a drug delivery strategy based on RME provides a promising cell permeation method (Fig. 1).[4] As a medicinal chemist, I am interested in drug membrane permeation. I have already described the drug delivery methods based on transporter-conscious drug design[3] and drug design using CPP[5] in other reviews. In this review, the possibility and implementation of drug delivery, particularly into cancer cells, using the RME system will be introduced.

Discussion

Receptor-mediated endocytosis

Most macromolecules are absorbed into cells through the endocytosis pathway rather than the transporter-mediated pathway and the passive diffusion pathway. There are many types of endocytosis (Table 1): (i) macropinocytic[6,7], (ii) micropinocytic[8], (iii) clathrin-dependent[9,10], (iv) caveolae-dependent[11], or (v) clathrin- and caveolae-independent[12]; based on differences in plasma membrane invagination formation (Table 1), and (i) receptor-initiated, (ii) non-receptor-initiated, or (iii) caveolae-initiated; based on differences in the trigger. Most receptor-mediated transport is clathrin-dependent. The binding of transported substances to such receptors induces clathrin-dependent endocytosis. Caveolae-mediated transport is involved in plasma invaginations composed of caveolins and lipid rafts. The trapping of transported substances in such invaginations induces caveolae-dependent endocytosis. From the standpoint of control of endocytosis, RME is available for delivery of substances.

Table 1
Categorization and features of endocytosis.

endocytosis	major factor	endosomal diameter	inhibitor	refs.
micropinocytosis		0.2–5 μm	EIPA, amiloride	6,7
micropinocytosis		< 0.1 μm		8
clathrin-dependent	clathrin	85–150 nm	chlorpromazine	9,10
caveolae-dependent	caveolin	50–100 nm	methyl-β-cyclodextrin	11
clathrin,caveolae-independent		ca.90 nm		12

However, in general, the precise mechanism of endocytosis, which depends on the conditions and characteristics of the transferred compound, remains unknown. The general mechanism is briefly described here. The binding of transported substances to the receptors invokes invagination of the plasma membrane to form endosomes, in which the transported substances are contained. Subsequently, the maturation from early endosomes (pH ca. 6.5) to late endosomes (pH ca. 5.5), and finally to lysosomes (pH ca. 4.5) is advanced by vacuolar H^+-ATPase proton pumps. During this maturation, the transported substances are thought to be considerably influenced by pH changes. The transported substances can escape from the endosome to cytoplasm by conformational or structural alterations; otherwise, they are degraded. Drug delivery using RME can take advantage of this successive endosome maturation to release the cargos out of the endosome. Furthermore, three strategies of anti-cancer payload delivery into cancer cells based on RME are proposed mainly based on using ligands such as (i) CPPs, (ii) tumor homing peptides, and (iii) monoclonal antibodies with affinity to the receptors on the membrane surface of cancer cells. With regard to these strategies, the size of the cargo is not of great concern due to endocytosis.

Design of ligand-payload conjugates through cleavable linkers or without covalent linkers

Trafficking of substances in cells after absorption across the membrane is very important for them to elicit their activity, because they are transiently trapped in the endosome. A fundamental principle in molecular design for drug delivery based on RME is the construction of components composed of payloads, ligands, and linkers, which connect the payload and the ligand (Fig. 1).

The linkers with which payload-ligand conjugates are tethered are

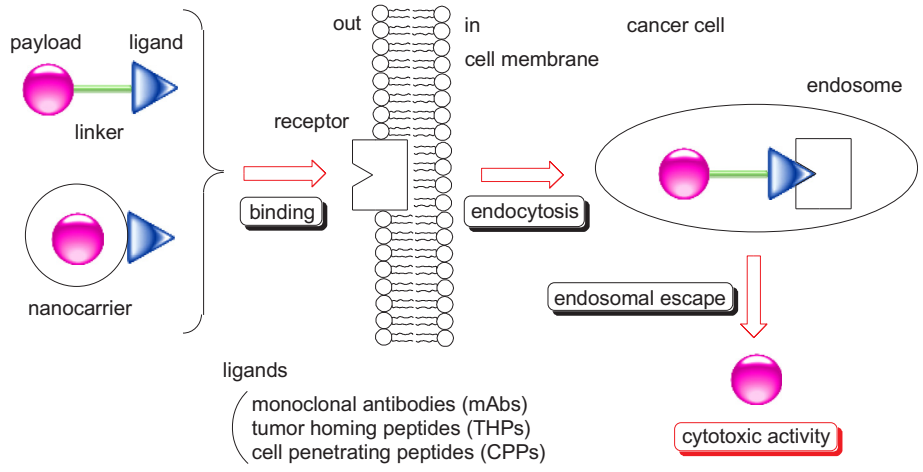

Fig. 1. Schematic strategy of selective substance delivery into cancer cells through receptor-mediated endocytosis.