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Sugar-modified poly(propylene imine) dendrimers as drug delivery agents for cytarabine to overcome drug resistance

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ABSTRACT

Maltose-modified poly(propylene imine) glycodendrimers (PPI-m OS) of the 4th generation provide a promising strategy for leukemia treatment. Anticancer therapy with nucleoside analog drugs such as cytarabine (Ara-C) frequently has limited efficacy due to drug resistance, inefficient uptake and accumulation of the drug inside cancer cells where it has to be transformed into the active triphosphate congener. The cationic nature of PPI dendrimers makes it possible to form complexes with nucleotide Ara-C triphosphate forms (Ara-CTP). The aim of this work was to test the concept of applying PPI glycodendrimers as drug delivery devices in order to facilitate the delivery of activated cytarabine to cancer cells to overcome metabolic limitations of the drug. The study has been carried out using 1301 and HL-60 leukemic cell lines as well as peripheral blood mononuclear cells. The results of cytotoxicity and apoptosis assays showed enhanced activity of Ara-C triphosphate form (Ara-CTP) complexed with PPI-m dendrimers in relation to free Ara-C and Ara-CTP against 1301 leukemic cells. Secondly, enhanced uptake and cytotoxicity of Ara-CTP-dendrimers complexes toward 1301 cells with blocked human equilibrative nucleoside transporter – hENT1 suggested that this combination might be a versatile candidate for chemotherapy against resistant acute lymphoblastic leukemia cells with lower expression of hENT1.

1. Introduction

Dendrimers are an interesting class of macromolecules with a number of potential applications in biology, pharmacy and biomedicine. Dendrimers are unique branched polymers of nanoscale size and well-defined globular shape (Biricova and Laznickova, 2009; Mintzer and Grinstaff, 2011). Their unique properties make them promising carriers for chemotherapeutic agents for cancer treatment (Medina and El-Sayed, 2009). It has been reported that dendrimers display higher stability than other carriers such as liposomes (Astruc et al., 2010). Moreover, a prevalent advantage of dendrimers is the ability to control their size and generation number. This results in the achievement of monodisperse macromolecules possessing defined surface groups after the synthetic process (Astruc et al., 2010).

The basic limitation of nanocarriers used in a cancer therapy arises from their toxicity. Ideal macromolecules used as a drug delivery system should be non-toxic and non-immunogenic (Nanjwade et al., 2009). Dendrimer toxicity strongly depends on the nature of their terminal groups. Cationic dendrimers are preferentially more cytotoxic and hemolytic than anionic or neutral macromolecules (Wolinsky and Grinstaff, 2008; Ziemba et al., 2012). In order to reduce cationic dendrimer toxicity, neutral molecules such as oligosaccharides (Appelhans et al., 2009;

Abbreviations: ALL, acute lymphocytic leukemia; AML, acute myeloid leukemia; Ara-C, cytarabine; Ara-CTP, cytarabine triphosphate; CDD, cytidine deaminase; dCK, deoxycytidine kinase; DP, dipyridamole; FBS, fetal bovine serum; hENT1, human equilibrative nucleoside transporter 1; NA, nucleoside analog; NBMPR, nitrobenzylmercaptopurine; OS, open shell; PBMC, peripheral blood mononuclear cells; PEG, polyethylene glycol; PHA-M, phytohemagglutinin; PI, propidium iodide; PPI, poly(propylene imine) dendrimers.

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Klajnert et al., 2008), polyethylene glycol (Astruc et al., 2010; Bhadra et al., 2003) or glycidol moieties (Wolinsky and Grinstaff, 2008) can be attached to the terminal groups.

Poly(propylene imine) (PPI) glycodendrimers modified with maltose residues are promising delivery vehicles for anionic chemotherapeutics such as nucleotide analogs (Fig. 1) (Szulc et al., 2015). Partial modification of terminal surface groups by maltose residues leads to lower toxicity when compared with unmodified PPI dendrimers (Klajnert et al., 2008; Ziemba et al., 2011, 2012, 2014). On the other hand, open shell (OS, partially surface-modified) PPI-m dendrimers are highly reactive macromolecules thanks to the presence of numerous internal and external cationic amino groups (Szulc et al., 2015). Thus, complexes with PPI glycodendrimers may allow for efficient delivery of the active form of the anionic drug directly to tumor cells.

Cytarabine (Ara-C), a member of a nucleoside analog (NA) group of anticancer drugs, is commonly used for the treatment of acute myeloid leukemia (AML), acute lymphocytic leukemia (ALL) and lymphomas (Ewald et al., 2008; Galmarini et al., 2001; Hamada et al., 2002; Robak and Robak, 2013). Ara-C is administered as an inactive prodrug (nucleoside form) and its passive diffusion across biological membranes is limited because of its hydrophilic nature (Galmarini et al., 2002a; Zhang et al., 2007). Like most NAs, Ara-C requires specialized equilibrative nucleoside transporter (ENT) proteins to cross plasma membranes or to be transported between intracellular compartments (Zhang et al., 2007). Cytarabine hENT1-mediated uptake is a necessary step to initiate its cytotoxicity (Zhang et al., 2007). Inside the cell, Ara-C is sequentially activated to a cytotoxic 5′-triphosphate form (Ara-CTP) by deoxycytidine kinase (dCK) and pyrimidine nucleotide kinases. Afterwards, the active (nucleotide) form of NA interferes with nascent nucleic acid synthesis and can be incorporated into DNA or RNA, and/or interfere with enzymes of nucleic acid metabolism, or modify physiological nucleoside/nucleotide metabolism leading to apoptosis of cancer cells (Ewald et al., 2008; Galmarini et al., 2001; Jordheim et al., 2013).

The effectiveness of clinically used anticancer drugs is also limited by various undesirable factors such as fast metabolism, unfavorable biodistribution, low specificity of interactions with cancer cells or a non-specific toxicity toward proliferating normal cells (Gouy et al., 2009; Hu et al., 2010; Jordheim et al., 2013). Moreover, the antitumor efficacy of Ara-C is frequently limited by several primary and acquired resistance mechanisms (Galmarini et al., 2010, 2002b; Jordheim et al., 2013; Jordheim and Dumontet, 2007). Primarily, the major factor in cytarabine resistance is associated with decreased expression of the human equilibrative nucleoside transporter 1 (hENT1) (Zhang et al., 2007). Furthermore, a decreased level of both dCK and other kinases, and an increased level of CDD (cytidine deaminase) are observed in resistant cells that leads to an inefficient uptake and accumulation of therapeutic agents inside cancer cells (Galmarini et al., 2001; Jordheim and Dumontet, 2007; Zhang et al., 2007). Secondly, cytarabine resistance may be caused by alterations of proteins that nucleoside analogs interact with to exert their cytotoxic effect, e.g. DNA polymerases, ribonucleotide reductase or CTP synthase (Galmarini et al., 2001; Jordheim and Dumontet, 2007). Eventually, the defective induction of apoptosis or modifications in the cellular response to changes induced by cytarabine may also be a cause of drug resistance (Galmarini et al., 2001; Jordheim and Dumontet, 2007).

In order to overcome some of the above-mentioned resistance mechanisms and to enhance cytarabine uptake into a cell and therapeutic effects, various cytarabine drug delivery devices have been studied (Hu et al., 2010). Encapsulation of cytarabine into liposomes was successfully developed in order to achieve a sustained release in palliative treatment of neoplastic meningitis

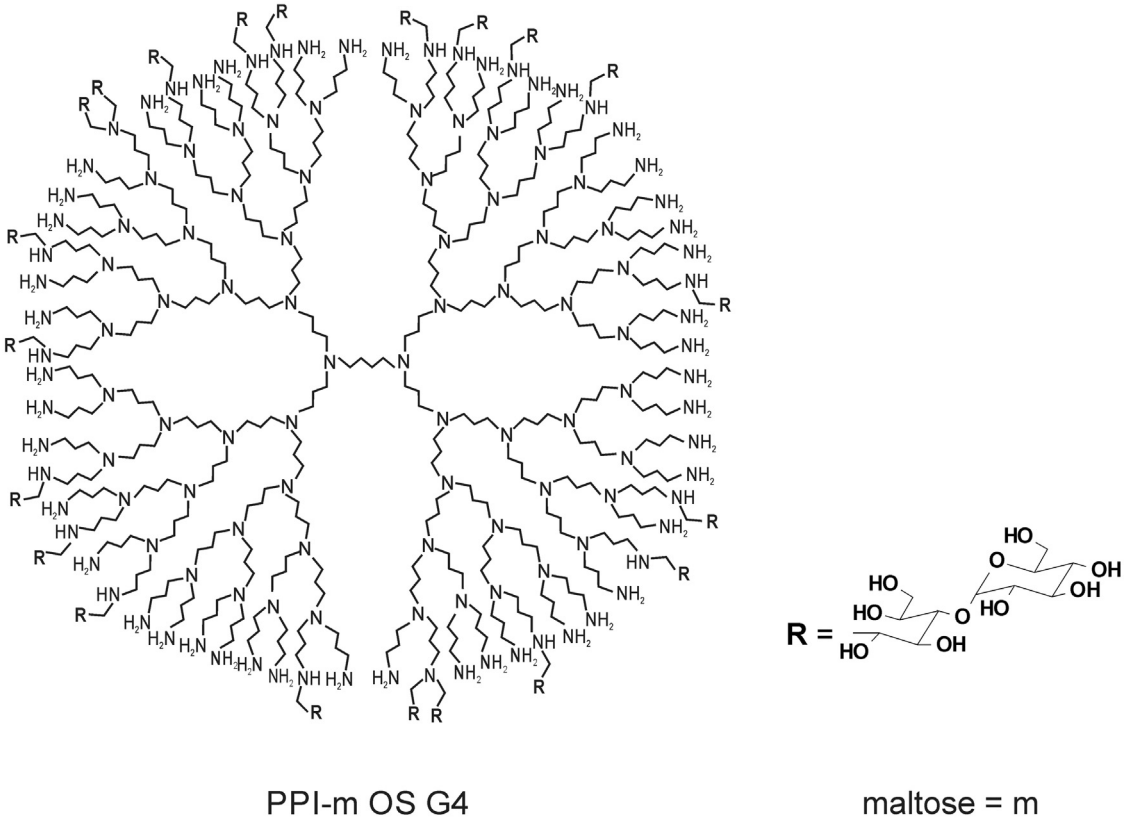

Fig. 1. The chemical structure of maltose-modified fourth generation poly(propylene imine) glycodendrimer.