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Development of a new LDL-based transport system for hydrophobic/amphiphilic drug delivery to cancer cells

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ABSTRACT

Low-density lipoproteins (LDL), a natural in vivo carrier of cholesterol in the vascular system, play a key role in the delivery of hydrophobic/amphiphilic photosensitizers to tumor cells in photodynamic therapy of cancer. To make this delivery system even more efficient, we have constructed a nano-delivery system by coating of LDL surface by dextran. Fluorescence spectroscopy, confocal fluorescence imaging, stopped-flow experiments and flow-cytometry were used to characterize redistribution of hypericin (Hyp), a natural occurring potent photosensitizer, loaded in LDL/dextran complex to free LDL molecules as well as to monitor cellular uptake of Hyp by U87-MG cells. It is shown that the redistribution process of Hyp between LDL molecules is significantly suppressed by dextran coating of LDL surface. The modification of LDL molecules by dextran does not inhibit their recognition by cellular LDL receptors and U-87 MG cellular uptake of Hyp loaded in LDL/dextran complex appears to be similar to that one observed for Hyp transported by unmodified LDL particles. Thus, it is proposed that dextran modified LDL molecules could be used as a basis for construction of a drug transport system for targeted delivery of hydrophobic/amphiphilic drugs to cancer cells expressing high level of LDL receptors.

1. Introduction

Level of selectivity of drug delivery to cancer cells is one of the most important factors that influence the efficiency of cancer treatment. Intravenously injected chemotherapeutics are transported throughout the body via the bloodstream and demonstrate promiscuous leak into extra-vascular space of both healthy and diseased tissues. Due to the drug excretion from the organism (Allen et al., 1995) and redistribution processes, the effective drug dose received by tumors is only a fraction of the dose administered. An increase of the amount of administered drug is not feasible for cytotoxic therapeutics, since it would cause major toxicity to healthy, non-cancerous cells. Thus, there is a critical need to find alternative delivery approaches to increase the effectiveness of tumor dosing and decrease dosing to normal tissues (Loomis et al., 2011). Various strategies have been investigated with the aim to develop suitable delivery system (polymer-, lipid-, metal- and carbon based nanoparticles), which assure a selective accumulation of drugs within the diseased tissue and the delivery of therapeutic concentration of drugs to the targeted site (Konan et al., 2002; Nishiyama et al., 2009; Danhier et al., 2010; Grund et al., 2011; Loomis et al., 2011; Serda et al., 2011). Stable and biocompatible delivery systems with a long circulation half-life in the blood are ideal. Additionally, achieving high specificity of delivery particles accumulation in the area of interest and release of drugs to their sites of activity is also very beneficial.

To provide selective delivery of drugs to the tumor tissue, design of delivery nano-particles that contains both drug and a tumor cell receptor-targeting moiety have been developed in order to reach: (i) high affinity of the binding moiety to the receptor on the targeted cell surface, (ii) increased efficiency and selectivity of drug delivery, and (iii) lower concentration of administered drug (Konan

Abbreviations: ApoB, apoprotein B; CCD, charge coupled device; DMEM, Dulbecco's modified Eagle medium; DMSO, dimethyl sulfoxide; EDTA, ethylene diaminetetraacetic acid; FBS, fetal bovine serum; FRET, Förster resonance energy transfer; Hyp, hypericin; LDL, low-density lipoproteins; LDL_b, BODIPY®FL stained low-density lipoproteins; MPS, mononuclear phagocytic system; PBS, phosphate-saline buffer; PDT, photodynamic therapy; Pts, photosensitizer; UV, ultraviolet; vis, visible.

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et al., 2002). As it is already shown by many groups, one of the possible approaches to reach these goals is to prepare delivery nano-particles on the basis of LDL (Firestone et al., 1984; Masquelier et al., 1986; Samadi-Baboli et al., 1993; Firestone, 1994; Versluis et al., 1996; Kader et al., 1998; Rensen et al., 2001; Kader and Pater, 2002; Zheng et al., 2005; Song et al., 2007).

LDL is known as the principal transporter of cholesterol in the human blood circulation system. LDL particles assume a globular shape with an average particle diameter 22 nm having three different regions. The outer surface layer, which consists of phospholipids molecules with a single apoB-100 protein wrapped around the surface of the LDL particle, the hydrophobic core of LDL which is composed of triacylglycerides and cholesterol esters, and an interfacial region between these two parts (Hevonoja et al., 2000; Prassl and Laggner, 2009). LDL have important advantages in comparison to other nano-delivery systems: (i) as natural molecules, LDL escape recognition by mononuclear phagocytic system (MPS) which favors their long circulation time in the plasma, (ii) they are not immunogenic, (iii) their hydrophobic core and phospholipid shell favor binding of hydrophobic and amphiphilic drugs, respectively. The importance of the study of drug–LDL complexes has substantially increased in the recent years since the US Food and Drug Administration encourage the inclusion of LDL–drug interaction studies as part of any new drug application that contains a hydrophobic compound (Wasan et al., 2008).

Photodynamic therapy (PDT), which involves systemic administration of photosensitizers (pts) followed by a local photoactivation of pts, is a promising method for the treatment of solid tumors (Wilson and Patterson, 2008). However, many pts used in PDT are hydrophobic or amphiphilic and cannot be simply injected intravenously. In general, moderately hydrophobic pts are preferentially transported in the bloodstream by albumins, whereas highly hydrophobic pts interact mainly with lipoproteins, especially with LDL (Jori, 1996; Reddi, 1997; Derycke and de Witte, 2004; Sharman et al., 2004). Thus, LDL could play a key role in the targeted delivery of hydrophobic and/or amphiphilic pts to tumor cells in PDT (Maziere et al., 1991; Jori and Reddi, 1993; Firestone, 1994; Polo et al., 2002; Jin et al., 2011). The targeted delivery of drugs complexed with LDL into tumor cells is possible due to the enhanced expression of specific LDL receptors in many types of transformed cancer cells relative to non-transformed cells (Brown and Goldstein, 1976; Shaw et al., 1987; Vitols, 1991).

Hyp (Fig. 1), (7,14-dione-1,3,4,6,8,13-hexahydroxy 10,11-dimethyl-phenanthrol [1,10,9,8-*opqra*] perylene), is a natural photosensitizing pigment from plants of the genus *Hypericum*. Hyp under light illumination causes anti-proliferative and cytotoxic effects (necrosis as well as apoptosis) in many tumor cell lines. These properties together with minimal dark toxicity, tumor selectivity and high clearance rate from the host body, make Hyp a promising agent for PDT of cancer as well as for tumor photodiagnosis (Shaw et al., 1987; Kiesslich et al., 2006; Kober et al., 2008; Miskovsky, 2002).

Our group has published several articles in the recent years about the properties of [Hyp–LDL] complex. We have shown that high Hyp/LDL ratios (>30/1) leads to a significant decrease of quantum yield of Hyp fluorescence (Kascakova et al., 2005). This decrease is caused by the formation of nonfluorescent Hyp aggregates inside LDL particles and by dynamic self-quenching of Hyp fluorescence (Mukherjee et al., 2008; Gbur et al., 2009). We have demonstrated the important role of the LDL receptor pathway for Hyp delivery into U-87 MG cells in the presence of LDL: a substantial increase of Hyp uptake was observed after overexpression of LDL receptors on the cell surface (Kascakova et al., 2008). It was also shown that overloading of LDL with Hyp (Hyp/LDL = 200/1) leads to a higher intracellular accumulation of Hyp molecules in comparison with the situation when the same quantity of Hyp is accumulated in LDL, but with a lower Hyp/LDL ratio (20/1). Moreover, Hyp/LDL ratio seems to affect the subcellular distribution of Hyp (Huntosova et al., 2010) and consequently the mechanism of photodynamically induced cell death.

The main goal of this work is to improve the effectivity and stability of LDL-based drug-delivery nanosystem by coating of LDL surface by dextran (Fig. 1). Dextran is a family of natural polysaccharides that is widely used as polymeric carrier in novel drug delivery systems. It is proposed that dextran prodrugs are very useful systems for achieving controlled drug release and drug targeting. Various dextran-antitumor drug conjugates enhances the effectiveness and improve the cytotoxic effects of chemotherapeutic agents (Varshosaz, 2012). However, in this work dextran serves as an element which modify surface of LDL particles. This modification should reduce the interaction of LDL with other serum constituents in order to prevent the redistribution of Hyp overloaded in LDL to the other free lipoproteins. Further, we would like to show that LDL surface modification by dextran does not influence the LDL recognition by LDL receptors of U-87 MG cancer cells as well as the cellular uptake of Hyp incorporated in LDL. This approach could lead to a construction of effective and selective delivery system of hydrophobic/amphiphilic drugs to cancer cells.

2. Materials and methods

2.1. Chemicals

Dextran T1, M.W. 1000 Da was purchased from PHARMACOSMOS (Denmark); LDL from human plasma was purchased from CALBIOCHEM (Germany); LDL_b (BODIPY®FL stained LDL) from human plasma, Hyp and PBS were purchased from Invitrogen (France); DMSO and Triton X-100 were purchased from Sigma–Aldrich (France); DMEM with GlutaMAX™, L-glucose (4500 mg L^{-1}) and sodium pyruvate (110 mg L^{-1}), penicillin/streptomycin and FBS were purchased from GIBCO® (France); Ultroser®G was purchased from Pall Corporation (France).

2.2. Cell culture

The U87-MG human glioma cells were grown in Dulbecco's modified Eagle's medium (DMEM) containing L-glutamine (862 mg L^{-1}), sodium pyruvate (110 mg L^{-1}), glucose (4500 mg L^{-1}), streptomycin (50 μg ml^{-1}), penicillin (50 μg ml^{-1}) and supplemented with 10% fetal bovine serum (FBS) or serum substitute 2% Ultroser G, in the presence of 5% CO_2 humified atmosphere at 37 °C. In all experiments DMSO concentration was under 0.5%. For all experiments, U-87 MG cells were incubated in dark conditions.

2.3. Preparation of dextran–LDL complex

100 μl of LDL solution (40 nM) in PBS pH 7.4 was prepared. Dextran powder was firstly diluted in PBS pH 7.4 and prepared in 900 μl PBS dextran solution at 37 °C. Such prepared dextran solution was carefully (100 μl by 100 μl) added to LDL solution and gently mixed. 1 ml of final 2.8 μM dextran/4 nM LDL (concentration ratio of dextran/LDL is 700/1) solution was kept at room temperature during 1 h for stabilization. For control experiments standard LDL was replaced by green fluorescence BODIPY®FL labeled LDL_b.

2.4. Preparation of dextran coated [Hyp–LDL] complex

Before the coating of LDL by dextran, [Hyp–LDL] complex was prepared. 0.8 μl of the stock solution of Hyp in DMSO (0.5 mM Hyp) was added to 100 μl of LDL (40 nM) in PBS solution (pH 7.4). The solution was then gently stirred and stabilized overnight at 8 °C