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Kinetics of incorporation/redistribution of photosensitizer hypericin to/from high-density lipoproteins

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ABSTRACT

By means of fluorescence spectroscopy we have studied the kinetics of interaction of a photosensitizer hypericin (Hyp) with high-density lipoproteins (HDL). Hyp is incorporated into HDL molecules as monomer till ratio Hyp/HDL ~8:1 and above this ratio forms non-fluorescent aggregates. This number is different from that found in the case of Hyp incorporation into low-density lipoprotein (LDL) molecules (8:1 vs 30:1). The difference is mainly attributed to the smaller size of HDL in comparison with LDL molecule. Biphasic kinetics of Hyp association with HDL was observed. The rapid phase of incorporation is completed within seconds, while the slow one lasts several minutes. The kinetics of the association of Hyp molecules with free HDL, Hyp/HDL = 10:1 complex and the redistribution of Hyp from Hyp/HDL = 70:1 complex to free HDL molecules reveal a qualitative similar characteristics of these processes with those observed for the interaction of Hyp with LDL. However, the incorporation of Hyp into HDL in the "slow" phase is more rapid than to LDL and extend of Hyp penetration into lipoproteins in the fast phase is also much higher in the case of HDL. The lower concentration of cholesterol molecules in outer shell of HDL particles is probably the determining factor for the more rapid kinetics of Hyp incorporation to and redistribution from these molecules when comparing with LDL particles.

1. Introduction

In the recent years an important attention has been dedicated to the use of lipoproteins for targeted drug delivery (Zheng et al., 2005; Lacko et al., 2007; Glickson et al., 2008; Ng et al., 2011). This fact is based on the establishing of a direct relationship between the relative number of lipoprotein receptors in various tumors and the uptake of drugs by malignant cells (Brown and Goldstein, 1976; Vitols et al., 1990; Puussinen et al., 2000; Damiano et al., 2013). Of the lipoproteins the most important in terms of drug delivery are low-density lipoproteins (LDL) (Konan et al., 2002; Polo et al., 2002; Sherman et al., 2004; Zheng et al., 2005; Derycke and de Witte, 2004), however, the importance of high-density lipoproteins (HDL) is also recognized and in many cases seems to be even higher than LDL (Corbin et al., 2007; Davis et al., 2008; McConathy et al., 2008; Damiano et al., 2013). The capacity of both types of the lipoproteins, LDL as well as HDL, to bind some drugs and their functionality as drug carriers have been examined in several studies (Jori, 1996; Lacko et al., 2002; Oda et al., 2006; Corbin et al., 2007). It has been also shown that mixing of anticancer drugs with LDL or HDL before administration led to an increase of cytotoxic effects of the drugs in the comparison when the drugs are administered alone (Jori and Reddi 1993; Chowdhary et al., 2003; Davis et al., 2008; McConathy et al., 2008).

LDL are recognized by and internalized into the cells through specific membrane receptors that interact with the apolipoprotein B of the LDL particle (Hevonoja et al., 2000). Certain tumor cells and tumor vascular endothelial cells express the LDL receptors in higher number due to either their increased proliferation or increased membrane turnover (Brown and Goldstein, 1976; Vitols et al., 1990). HDL have been implicated in cholesterol delivery in some malignancies including breast cancer, ovarian cancer, adrenocortical tumors and prostate cancer. The mechanism for HDL mediated delivery appears to be SR-B1 receptor dependent (Damiano et al., 2013). It is known that

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this receptor is broadly expressed among a variety of cancer cells (Rao et al., 2003). The above mentioned facts make LDL and HDL particles attractive natural occurring vehicles for drug delivery and targeting to cancer tissues.

Both types of the lipoproteins assume a globular shape with an average particle diameter 20–25 nm and 7–13 nm for LDL and HDL, respectively. These lipoproteins posses the outer surface layer, which consists of cholesterol and phospholipids molecules with a single apo B-100 protein wrapped around the surface of the LDL particle and apoA1, which is responsible for scaffolding, the size and shape of natural HDL, further the hydrophobic core composed of triacylglycerides and cholestrol esters, and an interfacial region between these two parts (Hevonoja et al., 2000; Prassl and Laggner, 2009; Jonas and Phillips, 2008). LDL and HDL have important advantages in comparison to other drug nano-delivery systems: (i) as natural molecules both lipoproteins 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 (Lacko et al., 2007; Glickson et al., 2008; Ng et al., 2011; Damiano et al., 2013). The importance of the study of the physicochemical properties of complexes of drugs with lipoproteins is confirmed by the fact that the US Food and Drug Administration (FDA) encouraged the inclusion of lipoprotein-drug interaction studies as part of any investigational new drug application that contains a hydrophobic compound (Wasan et al., 2008).

Our group has published in the recent years several articles about the properties of the complexes of LDL with photosensitizer hypericin (Hyp). Hyp (Fig. 1), (7,14-dione-1,3,4,6,8,13-hexahydroxy10,11-dimethyl-phenanthrol[1,10,9,8-*opqra*] perylene), is a natural photosensitizing pigment from plants of the genus *Hypericum*. This compound under light illumination causes antiproliferative and cytotoxic effects (necrosis as well as apoptosis) in many tumor cell lines. These properties, together with minimal dark toxicity, certain tumor selectivity, and high clearance rate from the host body, make Hyp a promising agent for photodynamic therapy (PDT) of cancer as well as for tumor photo-diagnosis (for reviews see Falk, 1999; Agostinis et al., 2002; Kiesslich et al., 2006; Karioti and Bilia, 2010). Chen et al. have shown that Hyp associates in human plasma predominatly with LDL and to a lesser extend with HDL and human serum albumin (HSA), however, binds dominantly to HDL in mouse plasma (Chen et al., 2001).

In our previous studies was 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 non-fluorescent Hyp aggregates inside LDL particles and by dynamic self-quenching of Hyp fluorescence (Mukherjee et al., 2008; Gbur et al., 2009). It was shown that only monomeric form of Hyp is able to produce the excited triplet state of Hyp in LDL, which in aerobic conditions leads to a singlet oxygen production (Gbur et al., 2009). Kinetics of Hyp association with free LDL molecules and Hyp/LDL complex have been also thoroughly studied and described (Huntosova et al., 2010; Buriankova et al., 2011). 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 over-expression 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.

To continue this study, we present a work in which a description of the association of Hyp with HDL particles is shown. The emphasis is given on the kinetics of Hyp incorporation into free HDL molecules, saturated Hyp/HDL = 10:1 complex, and the redistribution of Hyp from the complex Hyp/HDL = 70:1 to free lipoprotein particles. Similarly to our study of Hyp/LDL complexes (Kascakova et al., 2005; Buriankova et al., 2011), we have utilized the fluorescence spectroscopy approach and the fact that molecules of Hyp form non-fluorescent aggregates in aqueous solutions and the Hyp aggregates can be formed also in lipoprotein particles at high local Hyp concentrations (Kascakova et al., 2005; Lavie et al., 1995; Siboni et al., 2002). The differences between characteristics of Hyp association with LDL and HDL are also discussed.

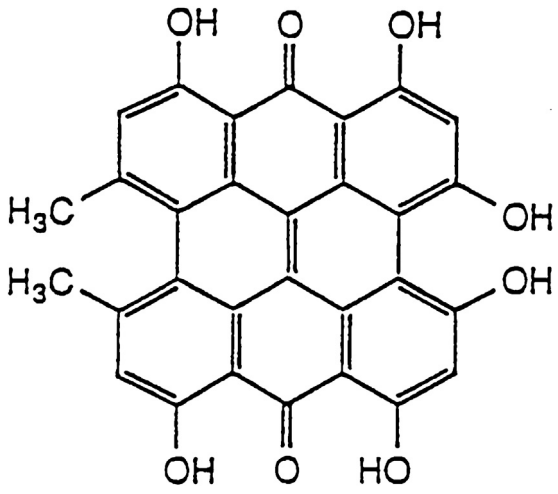

Fig. 1. Structure of hypericin.

2. Material and methods

2.1. Chemicals

Hypericin, ethylenediaminetetraacetic acid (EDTA), phosphate buffer saline (PBS) and dimethyl sulfoxide (DMSO) were obtained from Sigma–Aldrich. HDL (purity >95% of total lipoprotein content by electrophoresis) was purchased from Calbiochem (Darmstadt, Germany).

2.2. Preparation of Hyp and HDL solutions

The stock solution of Hyp was prepared by dissolving Hyp in 100% DMSO (c(Hyp) = 2 mM) and was kept in the dark at 4 °C. A stock solution of HDL (c(HDL) = 8.6 μM) was prepared in 150 mM NaCl solution at pH 7.4 in the presence of 0.1% EDTA. The Hyp–HDL solutions used in experiments were prepared by mixing of appropriate volumes of HDL and Hyp stock solutions in PBS at pH 7.4. The final concentration of DMSO in all Hyp–HDL solutions was under 1%. As revealed by UV–vis absorption and fluorescence spectroscopies, Hyp/HDL complexes are stable in PBS (pH 7.4) at room temperature (23 °C) for several days (5 days in this case).

2.3. Hyp incorporation into free HDL molecules

HDL solutions were mixed with Hyp as it is mentioned above. The concentration of HDL was kept at the constant value (10 nM). The final concentration of Hyp in Hyp/HDL complex varied from 10 nM to 700 nM (correspondingly Hyp/HDL ratio varied from 1:1 to 70:1).