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Deeper insights into the drug defense of glioma cells against hydrophobic molecules

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ABSTRACT

By means of fluorescence microscopy the intracellular distribution of fluorescent drugs with different hydrophobicity (quinizarin, emodin and hypericin) was studied. Selective photoactivation of these drugs in precisely defined position (nuclear envelope) allowed moderately hydrophobic emodin enter the nucleus. Highly hydrophobic hypericin was predominantly kept in the membranes with no fluorescence observed in the nucleus. The redistribution of quinizarin, emodin and hypericin between lipids, proteins and DNA was studied in solutions and cells. Based on these results was proposed theoretical model of hydrophobic drugs' nuclear internalization after photo-activation. Molecular docking models showed that hypericin has the strongest affinity to P-glycoprotein involved in the cell detoxification. Presence of 10 μM quinizarin, emodin or hypericin increased P-glycoprotein function in U87 MG cells. Moreover, emodin pretreatment allowed quinizarin nuclear internalization without photo-activation, which was not the case for hypericin. The synergy of such pretreatment and photo-activation should lessen the drug doses with simultaneous increase of drug efficacy triggering cell apoptosis/necrosis.

1. Introduction

The hydrophobicity of anticancer drugs and concept of their transport into targeted tissue is of interest for numerous cancer treatments. For the efficient transport drugs must be hydrophobic enough to enter the lipid bilayer, but they should also be able to be released from the membranes (Kubinyi, 1979). Hence, drug hydrophobicity plays a key role in its distribution, metabolism and clearance from the body (Eisenberg and McLachlan, 1986; Miyamoto and Kollman, 1993). Hypericin, quinizarin and emodin are naturally occurring plant pigments that possess anti-viral, anti-tumoral and anti-inflammatory properties (Andersen et al., 1991; Ma et al., 2003; Mueller et al., 1998; Nemeikaite-Ceniene et al., 2002; Quinti et al., 2003). These planar, aromatic organic compounds are redox catalysts and play an important role in many processes, *e.g.* electron transport, photosynthesis or cellular respiration (Bogdanska et al., 1999). Their influence on cell death pathways has been widely studied either in dark condition or after light-activation (Andersen et al., 1991; Huntosova et al., 2012; Mikesova et al., 2013; Mueller et al., 1998; Rossi et al., 2010). Also their molecular interactions with DNA were already studied (Miskovsky et al., 1995b; Saito et al., 2012; Verebova et al., 2014), to our knowledge no direct evidence of nuclear localization has been reported yet. The nuclear envelope (NcE) is considered to be the main barrier that protects nuclear DNA from interaction with anticancer drugs. Another problem for cancer treatment effectiveness is multi-drug resistance accompanied by decrease of drug accumulation in the resistant cells, due to active efflux of these drugs mainly by P-glycoprotein (P-gp) (Gottesman et al., 2002; Gottesman and Pastan, 1993). Photochemical activation at specific point/organelle within cells (Weyergang et al., 2015) is one of the possibilities how to overpass drug-resistance of the cancer cells. The second choice could be moderate regulation of P-gp activity as it was demonstrated in a wide variety of human cancer cells,

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including glioblastoma (U87 MG) (Kondo et al., 1996; Regina et al., 2001; Rittierodt et al., 2004). The role of P-gp is to detoxify cells by exporting chemically unrelated toxins (Aller et al., 2009). In this context and based on our previous works (Miskovsky et al., 1995a, 1995b; SanchezCortes et al., 1996), we evaluated using various experimental and theoretical approaches to both analyze distribution pattern for selected organic compounds as well as set up methodologies with aim to increase the drugs intracellular uptake and nuclear targeting. We have demonstrated that increase of the intracellular concentration of the small hydrophobic molecules takes place either by regulation of P-gp activity or by selective light-activation of these photosensitive molecules. This would importantly influence the targeting and induction of cell death pathways, apoptosis/necrosis.

2. Material and methods

2.1. Chemicals

Hypericin (Sigma-Aldrich, Germany) was dissolved in dimethyl sulfoxide (DMSO, Sigma-Aldrich, USA) to a final concentration of 2 mM. Emodin (Sigma-Aldrich, Germany) and quinizarin (Sigma-Aldrich, Germany) were dissolved in DMSO to the final concentration of 10 mM. Albumin from human serum (HSA, Sigma-Aldrich, USA) and low-density lipoproteins extracted from human serum (LDL, Invitrogen, Life science, France) were dissolved in 0.9% isotonic aqueous solution of NaCl (0.9% NaCl, Braun, Germany) to the concentration of 10 nM. Deoxyribonucleic acid sodium salt from calf thymus (Sigma-Aldrich, Germany) was dissolved at concentration of 2.3 mM in TRIS (Sigma-Aldrich, USA) buffer at pH 7.4. Anti-P Glycoprotein antibody (ab129450, Abcam, United Kingdom) and anti-PKCα pThr638 antibody (E195ab, Novus Europe) were used as primary antibodies for immunohistochemistry. Alexa Fluor®488 (LifeTechnologies, France) was used as secondary antibody. A nuclear probe Hoechst 33342 (Hoechst, Sigma-Aldrich, Germany) was dissolved in aqueous solution to 1.6 μM concentration. Solution of 100% methanol (Merck, Germany) was used for cell fixation. For all measurement the final content of DMSO was less than 1%.

2.2. Cell culture

U87 MG human glioma cells (Cells Lines Services, Germany) were plated and maintained according to propagation protocols onto 35 mm culture dishes with integral No. 0 glass cover slip bottoms (MatTek, USA). The U87 MG cells were grown in Dulbecco's modified Eagle medium (D-MEM), containing L-glutamine (862 mg/L), sodium pyruvate (110 mg/L), glucose (4500 mg/L), streptomycin (50 μg/mL) and supplemented with 10% fetal bovine serum (FBS), in the presence of 5% CO_2 hummified atmosphere at 37 °C. All chemicals were purchased from Gibco-Invitrogen, Life Technologies Ltd. Cells were incubated in the dark. After reaching 40–50% confluence, cells were used in experiments according protocols described below.

2.3. Intracellular distribution of drugs

The U87 MG cells were treated with 10 μM hypericin, emodin or quinizarin for 24 h. The concentration 10 μM was chosen with the aim to increase the fluorescence intensity and resolution of the micro-images. This concentration of the studied molecules is able to oversaturate the organelles in such manner, that their fluorescence is partially quenched by an aggregation process within these organelles (the process is explained in Huntosova et al., 2010), the free molecules in the cytoplasm can pass into the nucleus and it does not induce rapid cellular death. The 24 h incubation time was in accordance with literature (Huntosova et al., 2012; Li et al., 2014). Such timing should enhance (maximize) the ability of the drugs to enter the nucleus with simultaneous prevention of the cells from rapid death.

In order to assess intracellular localization pattern in U87 MG cells, experiments were performed under two different conditions. In the first case, exponentially growing cells were treated in the presence of drug (10 μM aqueous solution of hypericin, emodin or quinizarin) for 24 h prior to detection. Alternatively, cells were first fixed in ice cold 100% methanol for 1hr at −20 °C and then incubated with drug (10 μM hypericin, emodin or quinizarin in distilled water) for 24 h at room temperature and in dark.

After incubation the cells were washed and assessed with a 63X oil objective (NA 1.46) of LSM 700 confocal fluorescence microscope system (LSM, Zeiss, Germany). Excitation of hypericin was performed with 555 nm solid laser and quinizarin was excited with 488 nm solid laser. The detection window was chosen 580–630 nm for hypericin and 500–630 nm for quinizarin and emodin. The power of the lasers was not higher than 10% of the total power. Fluorescence signals were analyzed by the ZEN 2011 software (Zeiss, Germany). The plot profiles from the region of interests were measured in Image J software (National Institutes of Health, Bethesda, Maryland).

The nuclear localization was validated with staining of native and fixed cells with a nuclear probe Hoechst at the excitation 405 nm and the fluorescence was collected in the spectral range 420–500 nm.

2.4. Theoretical modeling of intracellular distribution and dynamics of drugs

The dynamics of intracellular distribution for hydrophobic drugs and their transport within the cells were analyzed using a top-down qualitative compartmental model (Patra and Klumpp, 2014). The model was proposed as a set of qualitative differential equations. The structure and parametric choices are still in a conceptual stage but they seek to integrate our qualitative knowledge about the observed system. Four compartments have been considered and denoted as *F* (free drug compartment), *O* (organelle compartment), *NcE* (nuclear envelope compartment) and *N* (nuclear compartment). The concentrations of the drug inside these compartments are denoted C_X with the transition rates k_{XY}, where *X and Y* can be substituted by *F, O, NcE* or *N*.

Redistribution of drugs between the compartments can be described with the phenomenological equations with the assumption that the levels of C_O,C_{NcE} and C_N are mediated by C_F. There was given assumption that concentration C_F plays a role of the common factor what means it has access to each compartment. Furthermore, the reversible kinetics can be expressed in four equations by general build up principles (Upadhay, 2006; Vriens, 1954) as follows:

$$dC_F/dt = J_F + k_{OF}C_O + k_{NcEF}C_{NcE} + k_{NF}C_N - C_F(k_{FO} + k_{FNcE} + k_{FN}) \quad (1)$$

$$dC_O/dt = k_{FO}C_F - k_{OF}C_O, \quad (2)$$

$$dC_{NcE}/dt = k_{FNcE}C_F - k_{NcEF}C_{NcE}, \quad (3)$$

$$dC_N/dt = k_{FN}C_N - k_{NF}C_N. \quad (4)$$

A balance between drug injection flow (administration of the drug into the system) J_F (of the extracellular origin) and intracellular transfer $dC_F/dt + dC_O/dt + dC_{NcE}/dt + dC_N/dt$ can be