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Macrophage selective photodynamic therapy by meta-tetra(hydroxyphenyl) chlorin loaded polymeric micelles: A possible treatment for cardiovascular diseases

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ABSTRACT

Selective elimination of macrophages by photodynamic therapy (PDT) is a new and promising therapeutic modality for the reduction of atherosclerotic plaques. m-Tetra(hydroxyphenyl)chlorin (mTHPC, or Temoporfin) may be suitable as photosensitizer for this application, as it is currently used in the clinic for cancer PDT. In the present study, mTHPC was encapsulated in polymeric micelles based on benzyl-poly(ε-caprolactone)-*b*-methoxy poly(ethylene glycol) (Ben-PCL-mPEG) using a film hydration method, with loading capacity of 17%. Because of higher lipase activity in RAW264.7 macrophages than in C166 endothelial cells, the former cells degraded the polymers faster, resulting in faster photosensitizer release and higher in vitro photocytotoxicity of mTHPC-loaded micelles in those macrophages. However, we observed release of mTHPC from the micelles in 30 min in blood plasma in vitro which explains the observed similar in vivo pharmacokinetics of the mTHPC micellar formulation and free mTHPC. Therefore, we could not translate the beneficial macrophage selectivity from in vitro to in vivo. Nevertheless, we observed accumulation of mTHPC in atherosclerotic lesions of mice aorta's which is probably the result of binding to lipoproteins upon release from the micelles. Therefore, future experiments will be dedicated to increase the stability and thus allow accumulation of intact mTHPC-loaded Ben-PCL-mPEG micelles to macrophages of atherosclerotic lesions.

1. Introduction

At present the world population encounters increase in age, obesity and lack of physical activity (Roger et al., 2012). This can give imbalance in the human physiology leading to among others high blood pressure, diabetes and unhealthy cholesterol levels, which eventually cause cardiovascular diseases being the number one cause of death in the western world. The prevalent contributor to cardiovascular morbidity is atherosclerosis, a chronic inflammation with slow progressive buildup of lipids and macrophages within the arterial wall (Libby et al., 2002). Lipid build up can start in the early thirties of a human's life and cause signs and symptoms 20 years later. Atherosclerosis is the main cause of myocardial infarctions and atherosclerotic plaque rupture.

When atherosclerosis is detected in an early phase, administration of hypolipidemic agents has mostly been applied as a therapy for the last two decades (Gotto and Pownall, 2015). More recently, novel therapeutic strategies involving treating vessel wall inflammation have emerged (Tabas and Glass, 2013). However, after administration of anti-inflammatory drugs there is a chance on the occurrence of side effects, primarily systemic immunosuppression and other off target

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effects (John and Kitas, 2012). When atherosclerosis is detected in a later phase it is mainly due to myocardial infarctions. In this stage the options are more rigorous and are mainly focused on opening the vessel by balloon angioplasty (Gray and Sullivan, 1996) with or without stenting (Farb et al., 1999) and in the most severe cases a bypass vessel (Sarjeant and Rabinovitch, 2002) will be installed. Currently much research is directed to repairing affected vessels by tissue engineered vessels that lack the drawbacks (unfavorable compliance and high stiffness) of current bypass vessels (Song et al., 2011a, 2011b). Until now none of these methods are actually treating or removing the inflammation and reoccurrence of myocardial infarctions in atherosclerotic patients is high (Davidsen et al., 2002; Smolina et al., 2012).

A new and promising combination therapy for the treatment of atherosclerotic plaques is photodynamic therapy (PDT) (Drakopoulou et al., 2011). A pivotal component of PDT in promoting plaque stabilization is sustained macrophage removal. Waksman et al. showed that PDT can reduce plaque formation and promotes smooth muscle cell repopulation if the macrophages are properly targeted (Waksman et al., 2008). Photodynamic therapy (PDT) is based on three interacting elements: 1) a non-aggregated photosensitizer (PS); 2) light of appropriate wavelength to activate the PS and 3) tissue oxygen (Henderson and Dougherty, 1992). When the PS is activated by light to the excited state and returns to the ground state, it releases energy, which is transferred to the surrounding tissue oxygen to generate singlet oxygen which in turn causes cell death (Guo et al., 2010). However, aggregation of PS molecules must be prevented, since the quantum yield of PS emission is highly dependent on its aggregation state (Ozoemena et al., 2001). Selective exposure of the plaque regions by light can be accomplished using an intra-arterial light emitting catheter (Jenkins et al., 1999). However, illumination of endothelial cell lining of the arteries that have possibly taken up PS can lead to atherosclerotic plaque rupture (Demidova and Hamblin, 2004), which should by all means be avoided. Therefore, selectively targeting the neo-vessels inside the plaques and/or the macrophages located in the plaques is highly desired. Importantly, most effective PS have low water solubility, therefore a simple intravenous injection is complicated and a safe solubilizing excipient or carrier system is necessary.

If one aims for PDT to become the future treatment of atherosclerosis, the problems stated above have to be overcome (Mulder et al., 2014). A method to circumvent these problems is by encapsulating the hydrophobic PS in nanoparticles. Besides enabling intravenous injection, nanoparticles are also known for enhancing therapeutic efficacy and safety of encapsulated drugs by improving solubility, ability of combining multiple drugs, protecting against metabolism, and controlling release (Mulder et al., 2014; Szelenyi, 2012). Furthermore, the formation of leaky vessels from the vasa vasorum in atherosclerotic plaques allows for accumulation of nanoparticles by the enhanced permeability and retention (EPR) effect inside the plaque and avoids uptake by the endothelium layer (Fang et al., 2011). Current research in atherosclerosis and nanoparticles primarily focuses on the targeted delivery of anti-inflammatory drugs (Lobatto et al., 2015; Sanchez-Gaytan et al., 2015; van der Valk et al., 2015), however such drugs usually only delay progression of atherosclerosis (Berman et al., 2013) thereby becoming a reoccurring medication during the lifetime of patients. In the present study we therefore combine the advantages of PDT and nanomedicine to develop a curative treatment for atherosclerosis.

Meta-tetra(hydroxyphenyl)chlorine (mTHPC) is a photosensitizer currently used in the PDT treatment of squamous-cell carcinoma (Lorenz and Maier, 2008) and available in a variety of commercial and EMA approved formulations including liposomes (Foscan®, FosLip®, FosPEG®). Previous research in our group showed that benzyl-functionalized micelles based on poly(ε-caprolactone)-*b*-methoxy poly(ethylene glycol) (Ben-PCL-mPEG) block copolymers can encapsulate mTHPC with very high loadings and remarkable stability (Carstens et al., 2007, 2008; Hofman et al., 2008). Most interestingly, it was shown that the release and subsequent photocytotoxic activity of mTHPC are controlled by lipase induced enzymatic degradation of the micelles (Hofman et al., 2008). This can be advantageous since it is known that macrophages present in the plaque synthesize lipoprotein lipase (LPL) (O'Brien et al., 1992), and therefore we hypothesized that it could thus potentially promote local release and activation of the photosensitizer once the loaded micelles are delivered to and internalized by the macrophages.

In this paper, mTHPC-loaded micelles were conveniently prepared by a film hydration method. We investigated if the LPL produced in macrophages can provide some cell specificity as compared to endothelial cells due to the enzymatic degradation of the micelles. The small size of the micelles might be advantageous for plaque penetration by the EPR effect. However, to selectively deliver the micelles to macrophages in the plaques, the micelles should at least be stable and retain their cargo during circulation. Therefore, we evaluated the in vitro stability in blood plasma and the in vivo pharmacokinetics of the mTHPC-loaded Ben-PCL-mPEG micelles.

2. Materials and methods

2.1. Materials

Acetonitrile, dimethyl sulfoxide (DMSO), trifluoroacetic acid, hydrogen peroxide, fetal bovine serum (FBS), trypsin, EDTA, PBS, ethanol, propylene glycol and high glucose Dulbecco modified eagle medium (DMEM) were obtained from Sigma-Aldrich (Zwijndrecht, the Netherlands). 384-Well plates were purchased from Fisher scientific GmbH (German). Optimem phenol red free was purchased at Invitrogen (Bleiswijk, the Netherlands). CellTiter 96® AQ_{ueous} One Solution was obtained from Promega (Leiden, the Netherlands). Human plasma and mice plasma were purchased from Seralab (UK) and mTHPC was purchased from Molekula (Germany). Foslip® (mTHPC liposomal formulation) and Foscan® (mTHPC in ethanol:propylene glycol 40/60 w/w) was kindly provided by Biolitec AG (Jena, Germany). Foslip® is composed of Phospholipids, glucose, and mTHPC with a dye: lipid ratio of ca. 1:13. Foslip® was reconstituted from lyophilized powder in distilled water. Foscan diluent is referred to a mixture of ethanol: propylene glycol (40/60 w/w).

2.2. Micelle formation

Micelles with and without mTHPC loading were formed by the film hydration method (Carstens et al., 2007). In short, 10 mg of Ben–PCL-mPEG block copolymer was dissolved in 1 mL of dichloromethane. Next, 5 mg of mTHPC was dissolved in 1 mL THF and different amounts corresponding to the desired polymer/photosensitizer ratios were mixed with the dichloromethane block copolymer solution. After evaporation of dichloromethane and THF in a vacuum oven, a thin solid film was formed. The formed film contained both block copolymers and photosensitizer that was visually distributed in a homogeneous way. The block copolymer (+ photosensitizer) film was subsequently hydrated in 1 mL PBS solution by gentle shaking and after 1 h subsequently filtered through a 0.2 μm syringe filter to remove non-encapsulated and thus precipitated photosensitizer. This procedure results in empty micelles or photosensitizer loaded micellar dispersions of 10 mg/mL Ben-PCL_n-$mPEG_{45}$. Size of micelles (by DLS) and critical micelle concentration were determined as described in Supplementary material.

2.3. Loading efficiency and capacity of micelles

The concentration of mTHPC in micellar dispersions was determined by UV/Vis spectroscopy. The dispersions were diluted in DMF to dissolve the micelles. A spectrum between 300 and 800 nm was recorded by a Shimadzu UV-2450 spectrophotometer (Shimadzu, Japan). The absorbance at 650 nm was analyzed against a calibration curve of