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The impact of porous silicon nanoparticles on human cytochrome P450 metabolism in human liver microsomes *in vitro*

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ABSTRACT

Engineered nanoparticles are increasingly used as drug carriers in pharmaceutical formulations. This study focuses on the hitherto unaddressed impact of porous silicon (PSi) nanoparticles on human cytochrome P450 (CYP) metabolism, which is the major detoxification route of most pharmaceuticals and other xenobiotics. Three different surface chemistries, including thermally carbonized PSi (TCPSi), aminopropylsilane-modified TCPSi (APTES-TCPSi) and alkyne-terminated thermally hydrocarbonized PSi (Alkyne-THCPSi), were compared for their effects on the enzyme kinetics of the major CYP isoforms (CYP1A2, CYP2A6, CYP2D6, and CYP3A4) in human liver microsomes (HLM) *in vitro*. The enzyme kinetic parameters, K_m and V_{max}, and the intrinsic clearance (CL_{int}) were determined using FDA-recommended, isoenzyme-specific model reactions with and without PSi nanoparticles. Data revealed statistically significant alterations of most isoenzyme activities in HLM in the presence of nanoparticles at 1 mg/ml concentration, and polymorphic CYP2D6 was the most vulnerable to enzyme inhibition. However, the observed CYP2D6 inhibition was shown to be dose-dependent in case of TCPSi and Alkyne-THCPSi nanoparticles and attenuated at the concentrations below 1 μg/ml. Adsorption of the probe substrates onto the hydrophobic Alkyne-THCPSi particles was also observed and taken into account in the determination of the kinetic parameters. Three polymer additives commonly used in pharmaceutical nanoformulations (Pluronics F68 and F127, and polyvinylalcohol) were also separately screened for their effects on CYP isoenzyme activities. These polymers had less effect on the enzyme kinetic parameters, and resulted in increased activity rather than enzyme inhibition, in contrast to the PSi nanoparticles. Although the chosen subcellular model (HLM) is not able to predict the cellular disposition of PSi nanoparticles in hepatocytes and thus provides limited information of probability of CYP interactions *in vivo*, the present study suggests that mechanistic interactions by the PSi nanoparticles or the polymer stabilizers may appear if these are effectively uptaken by the hepatocytes.

1. Introduction

Engineered nanoparticles are increasingly used in a variety of medical applications, including bioimaging, disease diagnoses, and drug delivery. Porous silicon (PSi) nanoparticles are a new class of nanocarriers used in drug delivery, and their feasibility for a range of medical uses is currently under evaluation (Salonen et al., 2008). The PSi nanoparticles have many advantages over the conventional drug carriers, including good biocompatibility both *in vitro* and *in vivo* (Bimbo et al., 2010; Korhonen et al., 2016; Nieto et al., 2013), the capability of enhancing the dissolution rates of poorly water-soluble payloads (Liu et al., 2013; Salonen et al., 2008; Santos et al., 2011), a high loading capacity (Liu et al., 2014; Salonen et al., 2005), and tunable surface chemistry (Huotari et al., 2013; Shahbazi et al., 2013; Shahbazi et al., 2014). Drug nanocarriers are by design intended for human use, thus their safety and toxicity to humans must be carefully evaluated prior to use. Common safety considerations of pharmaceutical nanoparticles typically address cytotoxicity, immune response, oxidative stress and production of reactive oxygen species, interactions with blood components, effects on the cardiovascular system, and biodegradation or accumulation in the body (De Jong and Borm, 2008; Naahidi et al., 2013). The influences of the nanoparticles on

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the molecular level biotransformation reactions of the co-administered drugs and other small molecular weight xenobiotics are however often neglected. For instance, the hepatic clearance of drugs may significantly change if the nanoparticles are capable of interacting with the enzymes that mediate drug detoxification, such as the hepatic cytochrome P450 (CYP) enzymes that are the major elimination pathway of drugs in humans (Anzenbacher and Anzenbacherová, 2001).

The amount of PSi nanocarriers administrated is largely dependent on their drug loading degree and the purpose of use. Although the doses of intravenously administered PSi nanoparticles are much lower than those of orally administered nanoparticles, these have been observed to accumulate in the liver and the spleen (Bimbo et al., 2010; Kallinen et al., 2014; Sarparanta et al., 2012; Wang et al., 2015a). However, the probability of the cellular uptake of PSi nanoparticles in hepatocytes is presently unknown. It also remains to be shown whether the PSi nanoparticles undergo vesicle transport into lysosomes or experience similar fate to drug molecules by interacting with the membrane-associated CYP enzymes located in the endoplasmic reticulum. Typically most nanoparticles are uptaken by the mononuclear phagocyte system, such as the Kupffer cells in liver or the macrophages in spleen, and it is known that both size and surface chemistry can impact the cellular uptake of nanoparticles in general (Huang et al., 2010). For instance, positive surface charge has been shown to favour uptake of gold nanoparticles by hepatocytes (Elci et al., 2016), and thus, also increase the risks of CYP interactions therein.

CYP-mediated metabolism is typically inhibited by competitive binding of the inhibitor to the enzyme's active site, or elsewhere on the enzyme to inhibit its activity noncompetitively or uncompetitively (Pelkonen et al., 2008). Particularly, the broad substrate specificity of CYP3A4 (metabolizes 50% of clinically used drugs) and the genetic polymorphism associated with CYP2D6 (metabolizes 30% of clinically used drugs) may result in significant changes in the efficacy and safety of the treatment (Ingelman-Sundberg et al., 2007), if these isoenzyme activities are affected by the exogenous inhibitors (such as nanoparticles). On the other hand, even minor CYP enzyme inhibition may lead to excessive (toxic) drug concentrations for drugs that have very narrow therapeutic range and only one elimination pathway. In addition to enzyme binding, nanoparticles may interfere with the microsomal lipid bilayers (Raesch et al., 2015), and thereby affect the activity of human CYPs embedded in the inner membrane of mitochondria or in the endoplasmic reticulum (Anzenbacher and Anzenbacherová 2001). Some xenobiotics may also induce CYP gene expression on a cellular level, and thus, accelerate the metabolism of a drug compound and result in too low (insufficient) drug concentration in the body (Berg et al., 2007; Pelkonen et al., 2008). CYP profiling and interaction screening *in vitro* are therefore mandatory steps in the early phases of drug discovery (FDA, 2014). Although prior studies have demonstrated that, for example, silver and gold nanoparticles may significantly affect CYP metabolism *in vitro* and *in vivo* (Midde and Kumar, 2015), the impacts of drug nanocarriers, such as PSi nanoparticles, have not been examined before.

This study focuses on the hitherto unaddressed effects of PSi nanoparticles on the human phase I metabolism in human liver microsomes (HLM) *in vitro* mediated by CYP3A4 and CYP2D6 (major drug toxifying isoenzymes), CYP1A2 (activation of, *e.g.*, carcinogenic polycyclic aromatic hydrocarbons (PAHs), and aflatoxin B_1) and CYP2A6 (endogenous and exogenous steroid regulation and detoxification of, *e.g.*, nicotine and polychlorinated biphenyls) (Anzenbacher and Anzenbacherová, 2001; McGraw and Waller, 2006; Pelkonen et al., 2008). The PSi nanoparticle types were chosen so that they have different surface properties (surface charge, hydrophobicity) and chemistries: thermally carbonized PSi (TCPSi), aminopropylsilane-modified TCPSi (APTES-TCPSi), and alkyne-terminated thermally hydrocarbonized PSi (Alkyne-THCPSi). As detailed information on the distribution of PSi nanoparticles in the liver does not exist, we focused on determining the CYP isoenzyme inhibition by using the HLM. Although the chosen model (HLM) is not able to predict the cellular disposition of PSi nanoparticles in hepatocytes and thus provides only limited information of probability of CYP interactions *in vivo*, it provides an established and well-characterized platform for mechanistic enzyme inhibition studies *in vitro*. In HLM, the CYPs are enriched, which provides a rapid approach to enzyme interactions screening besides the human liver cell lines, which may lose their liver-specific functions and thus show limited CYP activity upon culturing as such (Guillouzo et al., 2007, Y. Liu et al., 2015). The inhibition effects of selected polymer stabilizers commonly used in nanoparticle formulations, namely polyvinyl alcohol (PVA) and block copolymers (Pluronics) F68 and F127 (Batrakova and Kabanov, 2008; Sahoo et al., 2002), were also examined, as these may induce lipid bilayer rearrangements and thus affect the CYP activity for their part if effectively uptaken long with the nanoparticles into the hepatocytes. The impacts of PSi nanoparticles and polymers on CYP metabolism in HLM were examined using two independent analytical techniques, namely liquid chromatography-mass spectrometry (LC-MS) for determining the kinetics of FDA recommended CYP model reactions with and without PSi, and high-throughput well-plate (luminescence) assays for determining the minimum inhibitory concentrations of PSi nanoparticles.

2. Methods

2.1. Materials and reagents

Acetonitrile, coumarin, dimethyl sulfoxide (DMSO), formic acid, furafylline, 6β-hydroxytestosterone (100 μg/ml in methanol), ketoconazole, magnesium chloride, methanol, 8-methoxypsoralen, β-nicotinamide adenine dinucleotide 2′-phosphate reduced tetrasodium salt hydrate (NADPH), paracetamol, phenacetin, polyvinyl alcohol, quinidine, testosterone, Trizma base and umbelliferone were from Sigma-Aldrich (Steinheim, Germany). Hydrochloric acid, potassium dihydrogenphosphate, and perchloric acid were from Riedel-deHaën (Seelze, Germany). Dipotassium hydrogenphosphate was from Amresco (Solon, Ohio, USA). Bufuralol was from Roche (Basel, Switzerland) and 1′-hydroxybufuralol from Toronto Research Chemicals (North York, ON, Canada). Pluronics F68 and F127 were from BASF (Florham Park, New Jersey, USA). Human liver microsomes (pooled from 20 donors, 20 mg/ml total protein content) were from Corning (Wiesbaden, Germany). Pierce BCA (bicinchoninic acid) Protein Assay Kit was from Thermo Fisher Scientific (Rockford, IL, USA). The P450-Glo™ CYP2D6 Assay kit was from Promega (Madison, WI, USA). Water was purified with a Milli-Q water purification system (Millipore, Molsheim, France).

2.2. Nanoparticle preparation

Monocrystalline boron–doped p^+-type Si ⟨100⟩ wafers with a resistivity of 0.01–0.02 Ω cm were used in the fabrication of the PSi nanoparticles. Multilayer PSi films were prepared by electrochemically etching the Si wafers in a 1:1 (v/v) aqueous hydrofluoric acid (38%)–ethanol electrolyte with a pulsed etching profile. Free-standing films were obtained by increasing the etching current to the electropolishing region. PSi films of three different surface chemistries were crafted: TCPSi (Salonen et al., 2005), APTES-TCPSi (D. Liu et al., 2015; Mäkilä et al., 2012), and Alkyne-THCPSi (Wang et al., 2015b). The size reduction of the obtained multilayer films to nanoparticles was accomplished by wet milling. The final size selection of the PSi nanoparticles was done by centrifugation (16,000*g*, 10 min).

2.3. Nanoparticle characterization

The porous properties of the produced nanoparticles were characterized by nitrogen adsorption/desorption measurements on a Tristar 3000 (Micromeritics Inc., USA) at 77 K. The specific surface area was calculated using the Brunauer–Emmett–Teller method, and the pore