Enhanced bioavailability of peptide YY using nanoporous silicon as a drug carrier

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ABSTRACT

In the present study, we have used nanoporous silicon microparticles as drug carriers to improve anti-obesity peptide YY3-36 (PYY) delivery. Three different surface chemistries were used and PYY plasma concentrations were analyzed after subcutaneous injections as a function of time. Sustained release of PYY was obtained with all the three surface chemistries, but with a thermally oxidized surface, 2.5-fold increase in bioavailability was observed. Terminal half-lives were also significantly changed from the initial 25 min of PYY solution up to 21 h indicating a strong influence of PSi carriers on the *in vivo* pharmacokinetics of PYY.

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Keywords: porous silicon, peptide, drug delivery, sustained release.

1 INTRODUCTION

Biomedical applications of inorganic nanomaterials have been actively investigated during the last years. Porous silicon (PSi) is one of those new promising materials possessing several attractive properties. It is biodegradable and considered to be safe material for drug delivery, as has been investigated *in vitro* and *in vivo*[1-3]. In addition, the pore size, specific surface area, pore volume, particle size and surface chemistry can all be easily tailored to obtain optimized functionality for desired purposes. Native PSi is unstable and is therefore typically stabilized and depending on the purpose of the application, PSi surface can be further modified accordingly. PSi has previously been investigated for example with small molecules for oral delivery aiming to improve the pharmacokinetics of poorly soluble drugs, such as indomethacin *in vivo* [4]. In addition, PSi has been applied for potential cancer chemotherapy utilizing for example oxidation triggered delivery of doxorubicin *in vitro* [5]. Several proteins or peptides have also been adsorbed on PSi surfaces, such as human serum albumin (HSA), papain and insulin and characterized *in vitro* [6-10]. Favorably, peptide loading onto PSi does not need stressful procedures, harming the molecules and leading to bioinactivation, which has been a problem with traditional particulate systems [11-13]. Both, the pore structure and surface chemistry have been shown to affect the nature of drug adsorption, dissolution and release *in vitro* [6-9, 14, 15].

Peptide YY (PYY) is an endogenous peptide belonging to the same peptide family with neuropeptide Y (NPY) and pancreatic polypeptide (PP). PYY is a gut peptide secreted by enteroendocrine L-cells of the gastrointestinal tract in response to caloric payload, consists of 36 amino acids. It was found originally from porcine intestinal is able to cross the blood brain barrier with an unsaturable mechanism [16, 17] PYY3-36 is the main circulating form of the two endogenous forms of PYY, the other one being PYY1-36. The effects of PYY3-36 on food intake have been investigated in rodents, primates and lately also in humans and the effects are mediated via NPY2 receptors, which are abundant in central nervous system, especially in hypothalamus [18-20]. In rodents, it has been shown several times that PYY administration leads to reduced food intake and body weight [21, 22]. In addition to appetite regulation, PYY3-36 seems to have also a role in fuel substrate partitioning and energy homeostasis [23]. In both, obese and healthy human subjects PYY3-36 has a reducing effect on food intake [24-26]. Due to these actions; the PYY system has been suggested to be a potential therapeutical target for treatment of obesity [27, 28].

Mainly in order to investigate its physiological functions, PYY3-36 has been delivered chronically via osmotic minipumps or infusions in several experimental set ups in laboratory animals [28, 29]. However, because of the promising characteristics of PYY for treatment of obesity, also other suitable delivery systems for PYY have been investigated. As an example, reversibly PEGylated PYY3-36 has been shown to lead to prolonged inhibition of food intake in mice [30]. In humans, intranasal delivery of PYY3-36 has been tested with obese adults [31]. However, the needed administration frequency was 3 times per day. Furthermore, with higher doses the test subjects reported nausea and no statistically significant weight loss were obtained with lower doses. Also a longer lasting clinical

trial has been conducted by Nastech Pharmaceutical Company Inc. (www.clinicaltrials.com) using intranasal PYY3-36 with a similar administration frequency to the previous study. Recently, PYY3-36 has been also combined with GLP-1 and the combination was administered per orally, with a delivery agent SNAC, but obtaining detectable plasma concentrations not longer than 2 hours [20]. Since all the delivery systems need repeated dosing, a sustained release system is still required.

Peptide delivery using nanostructured PSi is not thoroughly investigated. Our recent *in vivo* studies with several different physiological parameters have indicated that nanostructured THCPSi microparticles can prolong the duration of action of peptides - ghrelin antagonist and melanotan II [32, 33]. To our knowledge, *in vivo* pharmacokinetic parameters of any peptide after delivery in nanostructured PSi and effects of different surface chemistries of PSi on those parameters have not been reported earlier.

In the present study, the effects of three different surface chemistries of nanostructured PSi microparticles on PYY3-36 delivery were investigated *in vivo*. Thermally oxidized PSi (TOPSi), thermally hydrocarbonized PSi (THCPSi) [34, 35] and a novel surface chemistry, THCPSi treated with undecylenic acid (UnTHCPSi) all sustained the release of PYY, but clear differences in pharmacokinetic parameters were found.

2 EXPERIMENTAL

2.1 Reagents

The silicon wafers were purchased from Cemat Silicon S.A. (Warsaw, Poland). Ethanol (99.5%) was bought from Altia (Helsinki, Finland). Hydrofluoric acid (HF) (37% – 39%) was acquired from Merck KGaA (Darmstadt, Germany). The nitrogen (99.999%) and the acetylene (99.6%) gases were bought from AGA (Espoo, Finland). Sodium chloride solution (9 mg/ml) for injections was obtained from B. Braun (Melsungen, Germany)HPLC reagents were acetonitrile (HPLC grade, JT Baker, Deventer, The Netherlands), trifluoroacetic acid (Sigma–Aldrich, St. Louis, MO, USA). pH 7.4, 0.15 M phosphate-buffered saline (PBS, μ = 0.167) was used as a buffer in the *in vitro* release experiments. The buffer contained 8.0 g sodium chloride (JT Baker Deventer, The Netherlands), 0.2 g potassium chloride (Merck KGaA, Darmstadt, Germany), 1.4 g disodium hydrogen phosphate (Merck KGaA, Darmstadt, Germany) and 0.2 g potassium dihydrogen phosphate (Merck KGaA, Darmstadt, Germany) in 1000 ml of deionized water.

2.2 Particle preparation

The preparation of free standing porous silicon films was performed as described previously [14. The free standing films were ball milled and dry sieved to a 38 – 53 µm size fraction. Aiming to remove any remaining small particles, after the dry sieving the particles were washed on a 38 µm sieve with ethanol. The microparticles were treated with a 1:1 HF:EtOH solution to replace the oxidized surface formed during the milling with a hydrogen termination and dried in 65 °C for 1 h. It should be noted that HF is highly corrosive and should be handled with caution.

The pore volume, average pore diameter and specific surface area of the PSi microparticles were calculated from desorption branch of the nitrogen sorption measurements (Tristar 3000, Micromeritics) according to BJH-theory [43]. The calculated values for TOPSi showed average pore diameter of 10.3 nm, specific surface area of 295 m^2/g and pore volume of 0.76 cm^3/g, for THCPSi; average pore diameter of 11.2 nm, specific surface area of 335 m^2/g and pore volume of 0.94 cm^3/g and for UnTHCPSi; average pore diameter of 10.4 nm, specific surface area of 294 m^2/g and pore volume of 0.76 cm^3/g, which slightly reduce during the surface treatments.

Thermal hydrocarbonization of PSi microparticles (THCPSi) was performed under continuous N_2/acetylene (1:1) flow as described earlier [35]. Treatment at 500 °C for 10 minutes was used in order to maintain partial hydrocarbon terminated and hence hydrophobic surface.

Functionalization of THCPSi microparticles was made by thermal treatments of the particles in undecylenic acid at 120 °C for 16h (UnTHCPSi) adapting the treatment for the hydrogen terminated PSi introduced by Boukherroub [44]. Due to the stressed and unsaturated carbon-carbon bonds existing on the hydrophobic surface of THCPSi particles immediately after the thermal hydrocarbonization, undecylenic acid covalently binds to the THCPSi surface and the treatment efficiency of about 50% can be achieved compared to the hydrogen terminated PSi (Supplementary material S1). This clearly changes the characteristics of the particles, such as drug loading and release properties, hydrophilicity and zeta potential. In addition, the carboxylic acid groups attached on the surface can be used for further functionalization of the particles. Thermal oxidation of PSi microparticles (TOPSi) was performed at 300 °C for 2 h in ambient air right after milling without any HF treatment. The treated particles were characterized using FTIR measurements (Spectrum BX II, Perkin-Elmer).

2.3 Drug loading

PYY3-36 was dissolved in water and the nanostructured PSi microparticles were immersed in the peptide solution (100 mg/ml) for 1.5 hours at room temperature. The loading solution was treated with ultrasound 3 times during the loading to ensure homogeneity. The particles were filtered from the solution and dried for 3 hours at room temperature and additional 30 minutes in vacuum. The loading degree was determined by thermogravimetric (TG) analysis (20

°C/min, 25 °C – 800 °C N_2 gas purge 200 ml/min, TGA 7, PerkinElmer) [45]. Peptide loading degrees of the batches were: THCPSi 12.2 %, TOPSi 15.2 % and 14.4 % and UnTHCPSi 16.0 % (w/w %) of drug in loaded particles) as measured by TG.

2.4 *In vivo* experiments

The Balc/C x DBA2 hybrid mice for the *in vivo* experiment were purchased from Lab Animal Center (Kuopio, Finland) at age of ~8 weeks, weighing 25 – 30g. They were group housed in a regulated environment; temperature 22 ± 1 °C, relative air humidity 55 ± 15 % and 12/12 hour light/dark cycle with lights on at 7 am. Commercial food pellets (Teklad 2016, Harlan Inc.) and tap water were available *ad libitum* throughout the experiment. The National Animal Experiment Board of Finland approved the experiments. Procedures were conducted in accordance with the guidelines set by the Finnish Act on Animal Experimentation (62/2006) and European Community Council Directives 86/609/EEC.

One of four different formulations, containing 20 µg of human PYY3-36 in 1) TOPSi 2) THCPSi 3) UnTHCPSi or 4) 0.9 % NaCl-solution were administered subcutaneously in a volume of 200 µl. Blood samples were collected from saphenous vein at predetermined time points into heparinized microcapillaries (Drummond Microcaps, Drummond Scientific Co. Broomall, Pa. USA). Plasma was separated by centrifugation and frozen immediately. Plasma samples were later analyzed using total human PYY3-36 ELISA following manufacturer's instructions (Millipore Corp., Billerica, MA, USA).

Pharmacokinetic parameters for PYY3-36 were determined from plasma concentration-time data by using WinNonlin software (WinNonlin Professional, 5.3, Pharsight Corp, USA) and non-compartmental analysis for extravascular administration, respectively. C_{max} and T_{max} – values were obtained directly from the plasma concentration –time data and area under the concentration time curve-values ($AUC_{0\text{-last}}$ and $AUC_{0\text{-}\infty}$) were determined by the trapezoidal rule.

Statistical differences of PYY3-36 plasma concentrations were analyzed using 2-way Anova with Bonferroni post-test for Multiple comparisons. Pharmacokinetic parameters analyzed using 1-way Anova with Bonferroni post-test and *in vitro* results were analyzed using Kruskal-Wallis (GraphPadPrism for Windows). Significant p-value was set <0.05.

3 RESULTS

All the three investigated PSi surfaces, TOPSi, THCPSi and UnTHCPSi, allow sustained PYY3-36 delivery over several days and the surface chemistry affects the rate and extent of PYY3-36 release *in vivo*. The non-covalently adsorbed drug is released from the nanopores of PSi microparticles by simple diffusion as the peptide microparticles are wetted by the solvent [33, 38].

In the present study, TOPSi microparticles significantly improve the relative subcutaneous bioavailability of PYY3-36. Yet the mechanisms behind the bioavailability improving character of TOPSi can only be speculated. Permeation enhancing feature of PSi might be one possible explanation for the improved bioavailability, since permeation improving properties have been earlier reported for PSi as has been investigated using Caco2-monolayers and furosemide or insulin loaded PSi and the latter with an additional permeation enhancer [10, 39]. On the other hand, peptide loading into nanosized pores seems to protect PYY3-36 from degradation before releasing from the particles. In addition, TOPSi might hinder peptide aggregation in the subcutaneous space, allowing total absorption of the peptide by controlling the release from the particles. Because it has been shown earlier that non-covalent peptide aggregation might form insoluble aggregates in subcutaneous formulations, which might lower the bioavailability tremendously [40].

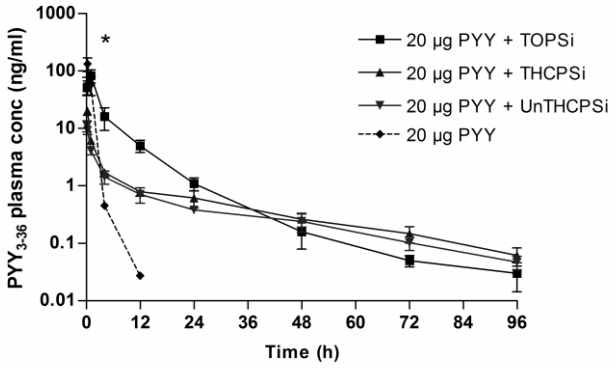

Figure 1. Sustained PYY3-36 release after delivery in nanostructured PSi microparticles is affected by surface chemistry of the microparticles (THCPSi and UnTHCPSi n=6, TOPSi n=5, n=4 for PYY3-36 solution, mean ± SEM). *TOPSi vs. THCPSi/UnTHCPSi p<0.001 0-4 h; p<0.005 TOPSi vs. THCPSi/UnTHCPSi at 4h.

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