



Research paper

Photocrosslinked poly(ester anhydride)s for peptide delivery: Effect of oligomer hydrophobicity on PYY3-36 delivery

Juha Mönkäre^{a,*}, Risto A. Hakala^{b,1}, Miia Kovalainen^a, Harri Korhonen^b, Karl-Heinz Herzig^{c,d}, Jukka V. Seppälä^b, Kristiina Järvinen^a

^aSchool of Pharmacy, University of Eastern Finland, Kuopio, Finland

^bPolymer Technology, School of Chemical Technology, Aalto University, Aalto, Finland

^cInstitute of Biomedicine and Biocenter of Oulu, University of Oulu, Oulu, Finland

^dDepartment Psychiatry, Kuopio University Hospital, Kuopio, Finland

ARTICLE INFO

Article history:

Received 25 May 2011

Accepted in revised form 15 September 2011

Available online 22 September 2011

Keywords:

Poly(ester anhydride)
Photocrosslinking
Peptide YY3-36
Controlled release
Bioavailability
Pharmacokinetics

ABSTRACT

The treatment for many diseases can be improved by developing more efficient peptide delivery technologies, for example, biodegradable polymers. In this work, photocrosslinked poly(ester anhydride)s based on functionalized poly(ϵ -caprolactone) oligomers were investigated for their abilities to achieve controlled peptide delivery. The effect of oligomer hydrophobicity on erosion and peptide release from poly(ester anhydride)s was evaluated by developing a sustained subcutaneous delivery system for an antiobesity drug candidate, peptide YY3-36 (PYY3-36). Oligomer hydrophobicity was modified with alkenylsuccinic anhydrides containing a 12-carbon alkenyl chain. PYY3-36 was mixed as a solid powder with methacrylated poly(ester anhydride) precursors, and this mixture was photocrosslinked at room temperature to form an implant for subcutaneous administration in rats. The oligomer hydrophobicity controlled the polymer erosion and PYY3-36 release as the increased hydrophobicity via the alkenyl chain prolonged polymer erosion *in vitro* and sustained *in vivo* release of PYY3-36. In addition, photocrosslinked poly(ester anhydride)s increased the bioavailability of PYY3-36 by up to 20-fold in comparison with subcutaneous administration of solution, evidence of remarkably improved delivery. In conclusion, this work demonstrates the suitability of photocrosslinked poly(ester anhydride)s for use in peptide delivery.

© 2011 Elsevier B.V. All rights reserved.

1. Introduction

Proteins and peptides are promising candidates for drug therapy in many therapeutic areas. However, they are still often formulated as suspensions or aqueous solutions that do not always allow the most efficient delivery of macromolecules [1]. Therefore, more sophisticated delivery systems need to be developed, i.e., to achieve sustained and controlled release [2,3] or protection against drug degradation at the site of administration [4,5].

Surface-erosion-controlled delivery systems hold great potential for peptide administration since the unreleased peptide remains intact inside the polymer matrix and the peptide release rate is directly proportional to the polymer erosion rate [6]. Recently, we have investigated photocrosslinked poly(ester anhydride)s for their potential in achieving surface-erosion-controlled drug delivery [7,8]. These biodegradable and biocompatible polymers combine

favorably the beneficial properties of the polyesters and polyanhydrides, such as good mechanical strength of polyesters [9] with the surface erosion property of polyanhydrides [10]. One of the advantages of these poly(ester anhydride)s is the flexibility offered by the possibility for the modification of oligomer chemistry. As an example, changing the oligomer hydrophobicity [11] can alter the polymer erosion and drug release rates. If one wishes to fabricate photocrosslinked poly(ester anhydride) devices, then the drug can be mixed as a solid powder with viscous liquid precursors at room temperature without the need for solvents, and by using a low-energy light source, one can avoid the unnecessary increase in the temperature. In addition, the drug loading degree and device dimensions can be varied extensively, and also injectable poly(ester anhydride) implants can be prepared.

Weight management is an example of a therapeutic application that could significantly benefit from more efficient peptide delivery technologies. Obesity is a growing global public health problem, and peptide YY3-36 (PYY3-36) is one of the most promising drug candidates for its treatment [12]. PYY3-36 is an endogenous gut hormone, secreted from L-cells in the gastrointestinal tract, which regulates energy metabolism in many ways, for example, by inducing satiety [12,13]. Previously, intravenous (i.v.) adminis-

* Corresponding author. School of Pharmacy, Faculty of Health Sciences, University of Eastern Finland, P.O. Box 1627, FI-70211 Kuopio, Finland. Tel.: +358 403553878; fax: +358 17162252.

E-mail address: juha.monkare@uef.fi (J. Mönkäre).

¹ These authors share equal contribution.

tration of PYY3-36 has been shown to reduce food intake in animals and humans [14–16] and to lower bodyweight in animals [17,18]. Due to its promising properties for the treatment of obesity, different delivery systems have been explored for PYY3-36, e.g., the intranasal [19] and peroral [20] routes. However, despite their many advantages, such as ease of dosing, these delivery systems have also disadvantages, such as evoking adverse effects or achieving inadequate therapeutic response. In a 12-week trial, intranasal administration before meals did not induce weight loss and a higher dose evoked adverse effects, such as nausea and vomiting [19]. The peroral route has been shown to be well tolerated but PYY3-36 only appeared to be able to reduce the caloric intake when administered with GLP-1 before a meal [20]. Interestingly, subcutaneous (s.c.) injections of PYY3-36 solution have been shown to increase the feelings of satiety and to decrease hunger in obese males with minimal adverse effects despite the presence of higher plasma concentrations than obtained after intranasal administration [19,21]. In addition, administration via the s.c. route produced an elevation of PYY plasma concentrations for more than 4 h, which was speculated to evoke a long-lasting appetite-suppressive effect [21].

In this work, the suitability of photocrosslinked poly(ester anhydride)s for controlled peptide delivery was evaluated by developing a sustained s.c. delivery system for PYY3-36 (M_w 4050 g/mol). The poly(ester anhydride) oligomers were functionalized with succinic anhydride (SAH) or with the more hydrophobic alkenylsuccinic anhydride (12-ASA) containing a 12-carbon alkenyl chain in order to clarify the effect of oligomer hydrophobicity on peptide delivery.

2. Materials and methods

2.1. Materials

Oligomers were polymerized from ϵ -caprolactone (Solvay Interox Ltd., Warrington, England) in the presence of stannous octoate and pentaerythritol. The ϵ -caprolactone was redistilled and dried over molecular sieves. Stannous octoate as an initiator and pentaerythritol as a co-initiator were used in the ring-opening polymerization of the oligomers. Succinic anhydride (SAH), 2-dodecylsuccinic anhydride (12-ASA), and methacrylic anhydride were used in the functionalizations, and camphorquinone was used as an initiator for photocuring. All reagents except ϵ -caprolactone were purchased from Sigma–Aldrich Chemie, Germany, and were used as received.

Human peptide PYY3-36 (PYY3-36, M_w 4050 g/mol) was purchased from BCN Peptides (Barcelona, Spain). In the *in vitro* drug release study, phosphate-buffered solution, pH 7.4 (0.2 M NaOH- KH_2PO_4) was prepared using NaOH from FF-Chemicals (Yli-Ii, Finland) and KH_2PO_4 from Merck (Darmstadt, Germany). Bovine serum albumin (BSA, Sigma–Aldrich, St. Louis, MO, USA) (0.1% w/v) was dissolved in the buffer in order to prevent the adsorption of PYY3-36 onto the laboratory materials during the *in vitro* experiment. Sodium chloride solution (9 mg/ml) for injections was obtained from B. Braun Melsungen AG (Melsungen, Germany) and Baxter Oy (Vantaa, Finland).

2.2. Animals

Male Wistar rats (age of 8–12 weeks, 293–398 g, mean 328 g) (Kuopio, Finland) were housed in an environment-controlled room temperature 21 ± 1 °C, relative air humidity $55 \pm 15\%$, and 12/12 h light/dark cycle with lights on at 7 AM with food (Teklad 2016, Harlan Inc.) and tap water available *ad libitum*. 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.

2.3. Preparation of poly(ester anhydride) implants

The synthesis and properties of the poly(ester anhydride) based on poly(ϵ -caprolactone) oligomers have been described earlier [11,22], and the main features are shown in Scheme 1. Briefly, ϵ -caprolactone monomers were polymerized to star-shaped hydroxyl telechelic oligomers by ring-opening polymerization. In the next step, the hydroxyl termination was changed to acid termination with succinic anhydride (SAH) or 2-dodecylsuccinic anhydride (12-ASA). In order to obtain crosslinkable poly(ester anhydride) precursors with labile anhydride bonds, acid-terminated oligomers were allowed to react with methacrylic anhydride.

In the photocrosslinking, the viscous liquid methacrylated precursors, camphorquinone (1% w/w) with and without PYY3-36 powder (1% w/w) were stirred until homogeneity was achieved and the mixture was then placed into a mold in order to produce the implants (2 mm in thickness, 5 mm in diameter, and 50 mg in weight). Photocrosslinking was done using visible light (11 W, 16 mW/cm²) at room temperature for 20 min. The mean PYY3-36 doses were 525 and 483 $\mu\text{g}/\text{implant}$ for SAH- and 12-ASA-functionalized implants, respectively.

The gel contents of the photocured implants were measured by extracting the soluble phase in dichloromethane at room temperature for 24 h, and it was found that gel contents were >95%. Attenuated total reflectance infrared spectroscopy (ATR-FTIR, Nicolet Magna-FTIR spectrometer 750 equipped with PIKE Technologies GladiATR with diamond crystal plate) was used to monitor the double bond conversion of the methacrylated precursors. The double bond conversion in the implants was calculated based on the decrease in absorbance near 1637 cm⁻¹, a characteristic absorbance of the methacrylate double bond [22,23]. The final double bond conversions were >93%.

2.4. *In vitro* erosion of implants

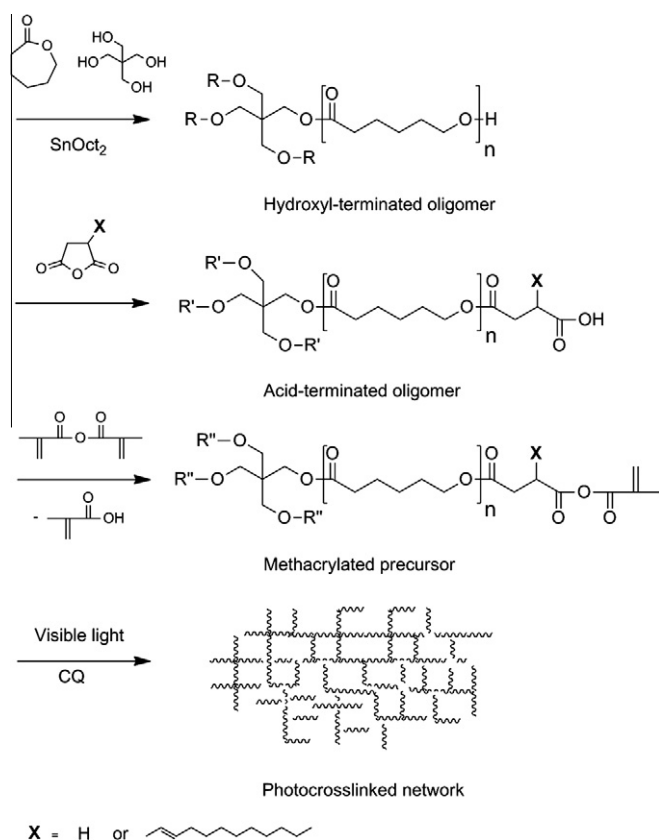
The *in vitro* erosion ($n = 3$) of the poly(ester anhydride) implants was studied in 40 ml of pH 7.4 phosphate buffer in the water bath shaker with orbital shaking at a frequency of 120 strokes/min at +37 °C (Grant OLS200, Cambridge, UK). Samples were removed from the buffer at predetermined time intervals and dried in a vacuum for 48 h prior to weighing. The erosion (%) was calculated by dividing the dried weight of the sample by its initial weight.

2.5. *In vitro* release of PYY3-36

The *in vitro* PYY3-36 release ($n = 3$) from the SAH- and 12-ASA-functionalized poly(ester anhydride) implants was studied by using USP Apparatus 1 (basket) under sink conditions. The buffer (pH 7.4, +37 °C) volume was 400 ml, and the rotation speed of the basket was 50 rpm (Sotax AT6, Sotax AG, Basel, Switzerland). Samples (2 ml) were collected at predetermined time intervals, and the sampling volume was replaced by fresh prewarmed buffer. The released PYY3-36 in the buffer was analyzed using total human PYY ELISA kit (Millipore Corp., Billerica, MA, USA) according to the manufacturer's instructions.

2.6. *In vivo* delivery of PYY3-36

One photocrosslinked poly(ester anhydride) implant was implanted subcutaneously in the back of each rat. In addition to the PYY3-36-loaded SAH- and 12-ASA-functionalized implants ($n = 6$), peptide-free SAH-functionalized ($n = 4$) and 12-ASA-functionalized ($n = 3$) implants were implanted and three rats were sham-operated



Scheme 1. Preparation of photocrosslinked poly(ester anhydride)s from star-shaped hydroxyl-terminated poly(ϵ -caprolactone) oligomers.

as controls. The implants were purified with ethanol/water (70:30) solution before the insertion. Prior to the implantation, the rats were anesthetized with a fentanyl citrate/fluanizone/midazolam mixture (0.158 mg/5 mg/2.5 mg/kg, s.c.). Blood samples were collected from the saphenous vein at predetermined time intervals into heparinized capillaries (Drummond Microcaps, Drummond Scientific Co. Broomall, Pa. USA). The plasma was separated by centrifugation and frozen immediately. The implantation site was inspected visually at the end of the experiment after sacrifice in order to visually detect any remainders of the implants. PYY3-36 plasma concentrations were analyzed using total human PYY ELISA kit (Millipore Corp., Billerica, MA, USA) according to the manufacturer's instructions. By using this ELISA kit, the administered human PYY3-36 in plasma was analyzed and endogenous rat PYY3-36 did not interfere with the results.

In order to characterize the pharmacokinetics of PYY3-36, peptide solution (200 μ l, 0.9% NaCl) was administered both i.v. and s.c. with doses of 2 and 20 μ g to the rats ($n = 4$). Blood samples were collected and analyzed as described above. In order to avoid adverse effects and the need for excessive dilution of plasma samples due to high plasma concentrations, lower doses were used in i.v. and s.c. administration of solutions than were present in implants.

2.7. Pharmacokinetic analysis

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 model for extravascular and bolus intravenous injection in the cases of s.c. and i.v. administration, respectively, and with uniform weighing. All data are presented as mean \pm SEM.

Maximum plasma concentration (C_{max}) and time to reach C_{max} values (t_{max}) were obtained directly from the plasma concentration–time data. Terminal half-life ($t_{1/2}$) was calculated as $0.693/K_e$, where K_e is the terminal elimination rate constant. The area under the concentration–time curve values (AUC_{0-last}) until the last measured plasma concentration (C_{last}) were determined by the linear trapezoidal rule, and $AUC_{0-\infty}$ was determined as $AUC_{0-last} + C_{last}/K_e$. Clearance (CL) and volume of distribution (V_d) were calculated as $D/AUC_{0-\infty}$ and $D/(K_e \cdot AUC_{0-\infty})$, where D is dose. Absolute bioavailability (F%) was calculated as $(AUC_{e.v.}/AUC_{i.v.}) \cdot (D_{i.v.}/D_{e.v.}) \cdot 100\%$, where $AUC_{e.v.}$ and $AUC_{i.v.}$ are $AUC_{0-\infty}$ values of extravascular and i.v. administration, respectively, and $D_{e.v.}$ and $D_{i.v.}$ are the corresponding doses for extravascular and i.v. administration. Relative s.c. bioavailability ($F\%_{rel}$) was calculated by using $AUC_{0-\infty}$ values and doses of s.c. solution (s.c.) poly(ester anhydride) (PEAH) administration as follows: $(AUC_{PEAH}/AUC_{s.c.}) \cdot (D_{s.c.}/D_{PEAH}) \cdot 100\%$. *In vivo* release and absorption profile based on percent AUC values ($AUC\%$) were determined as $(AUC_{0-t}/AUC_{0-\infty}) \cdot 100\%$ [24].

2.8. Statistical analysis

Mann–Whitney U test was used to compare the statistical differences of PYY3-36 between pharmacokinetic parameters of two groups (i.v., s.c., or implant administration) (GraphPadPrism 5.03 for Windows, GraphPad Software, San Diego, CA, USA, www.graphpad.com). Kruskal–Wallis (SPSS 14.0 for Windows, SPSS Inc.) with *post hoc* test [25] was used to compare statistical differences between multiple groups. The level of significance was set at $p < 0.05$.

3. Results

3.1. *In vitro* erosion of implants and PYY3-36 release

The increase in oligomer hydrophobicity by the introduction of alkenylsuccinic anhydride with 12-carbon alkenyl chain (12-ASA) slowed down the erosion of photocrosslinked peptide-free poly(ester anhydride) implants (Fig. 1). 12-ASA-functionalized poly(ester anhydride) implants eroded in 72 h and the erosion rate was $1.7 \pm 0.01\%/h$ with a 12 h lag time. Peptide-free SAH-functionalized poly(ester anhydride) eroded in 48 h with the rate of $2.0 \pm 0.04\%/h$ without any lag time, as reported earlier [8]. Irrespective of the degree of hydrophobicity, both poly(ester anhydride) implants exhibited surface-eroding characteristics.

The *in vitro* release of PYY3-36 was also affected by the oligomer hydrophobicity. PYY3-36-loaded SAH-functionalized implants released PYY3-36 completely *in vitro* in 24 h showing a linear release rate of $4.5 \pm 0.33\%/h$ without any lag time (Fig. 2 and Table 1). However, the fraction of PYY3-36 released from 12-ASA functionalized *in vitro* was less than 20% in 60 h, although implants were completely eroded within that time (Fig. 2). One explanation for this result could be that poorly soluble degradation products of 12-ASA-functionalized poly(ester anhydride) had been deposited on the implant surface and PYY3-36 was interacting with this film-like layer [26,27].

3.2. *In vivo* delivery of PYY3-36

I.v. administration of 2 and 20 μ g of PYY3-36 exhibited linear pharmacokinetics of PYY3-36, based on first-order elimination kinetics as presented in Table 2 and Fig. S1 of Supplementary data. The terminal elimination half-lives ($t_{1/2}$) of PYY3-36 were approximately 40 min, which is slightly longer than earlier reported values for mice (13 min) [28] or rabbits (13–19 min) [17]. The AUC values achieved with 2 μ g and 20 μ g i.v. doses of PYY3-36 were compared, and the difference in values was only 6-fold instead of the expected

10-fold increase, based on the assumption of linear pharmacokinetics. A comparison of primary pharmacokinetic parameters between doses showed that values for volume of distribution (V_d , $p < 0.05$) and clearance (CL) were smaller after 2 than after 20 μg dose. However, the ratio of V_d to CL remained constant, and therefore, changes did not affect the terminal half-lives.

S.c. administration of PYY3-36 solution displayed evidence of limited s.c. absorption of PYY3-36. The absolute bioavailability ($F\%$) values were low and dose-dependent, being $17.9 \pm 6.2\%$ and $6.3 \pm 1.1\%$ for 2 and 20 μg doses, respectively (Table 2). Furthermore, the difference between $AUC_{0-\infty}$ values of 2 and 20 μg doses was only 1.8-fold, indicating that the extent of s.c. absorption of PYY3-36 declined as the dose increased. In contrast, the dose did not affect significantly the absorption rate, i.e., t_{max} and the shape of plasma concentration–time profile (Table 2, Fig. S1). Elimination half-lives after s.c. administration were 42 ± 4.2 min and 78 ± 24.0 min for 2 and 20 μg doses, respectively, that are comparable to the literature values for non-parenteral administration in humans (per oral 24–50 min) [29] or rabbits (intranasal 34–40 min) [30].

In contrast to s.c. administration of PYY3-36 solution, PYY3-36 was absorbed nearly completely into the systemic circulation from the photocrosslinked poly(ester anhydride) implants (Table 2). The bioavailabilities of PYY3-36 administered in SAH-functionalized or 12-ASA-functionalized poly(ester anhydride) implants were $81 \pm 13.2\%$ ($131 \pm 21.5\%$) and $71 \pm 20.4\%$ ($116 \pm 33.1\%$) when compared with 2 μg (or 20 μg) i.v. doses, respectively (Table 2). Significant differences ($p < 0.05$) between absolute bioavailabilities of 20 μg s.c. solution and poly(ester anhydride) implants were found. Furthermore, the relative s.c. bioavailabilities ($F\%_{\text{rel}}$) of PYY3-36 from the photocrosslinked poly(ester anhydride) implants ranged from 330% to 2070%, which indicates that there was 3- to 20-fold higher absorption from implants than from s.c. solution injection (Table 2). These results demonstrate that photocrosslinked poly(ester anhydride)s can significantly improve PYY3-36 delivery in comparison with the solution administration.

PYY3-36 administration via s.c. implanted photocrosslinked poly(ester anhydride)s significantly sustained the release of the peptide. After administration in solution, PYY3-36 was detected in plasma for only 4 h, whereas peptide administration via poly(ester anhydride) implants prolonged the detection period up to 9 days (Fig. 3). The duration of the *in vivo* release was analyzed by the percent AUC method [24], which describes the drug input into systemic circulation by combining release and absorption phases. The comparison of SAH-functionalized and 12-ASA-func-

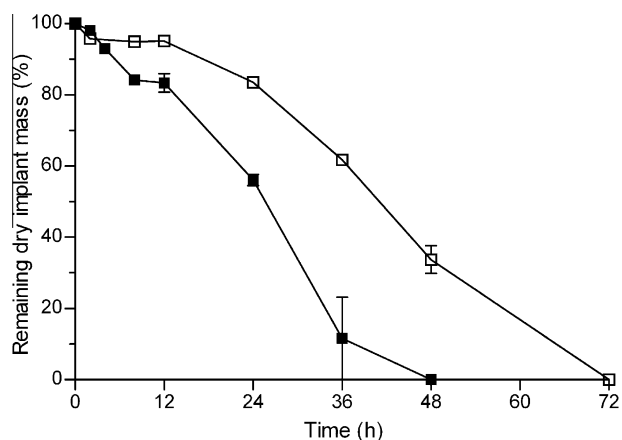


Fig. 1. *In vitro* erosion of peptide-free SAH- (■) and 12-ASA-functionalized (□) photocrosslinked poly(ester anhydride) implants in pH 7.4 buffer (+37 °C) (mean \pm SEM, $n = 3$).

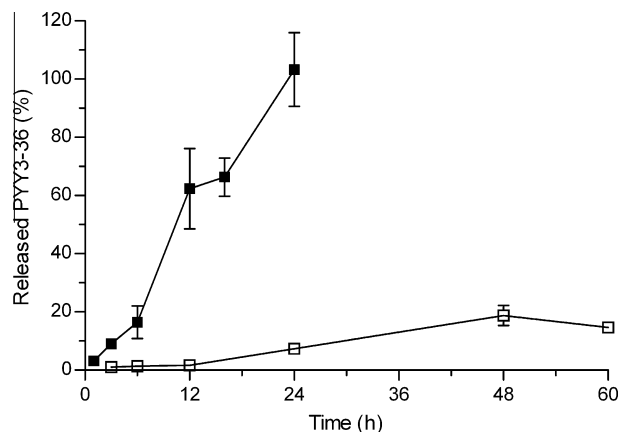


Fig. 2. PYY3-36 *in vitro* release from SAH- (■) and 12-ASA-functionalized (□) photocrosslinked poly(ester anhydride) implants in pH 7.4 buffer (+37 °C) analyzed using total human PYY ELISA kit (mean \pm SEM, $n = 3$).

tionized implants indicated that hydrophobic modification with 12-carbon alkenyl chain sustained PYY3-36 release from 3 to 7 days (Fig. 3). In addition, the linear *in vivo* release rate halved from $2.0 \pm 0.10\%/h$ of SAH-functionalized to $1.0 \pm 0.07\%/h$ with the 12-ASA-functionalized implants. Similarly, oligomer modification with 12-ASA more than doubled the duration of the linear release phase from 46 to 102 h and prolonged the lag time from 22 ± 2.0 to 38 ± 4.8 h (Table 1). In addition, pharmacokinetic parameters describing the rate of absorption were affected by the oligomer modification, so that t_{max} was delayed from 42 ± 4.0 to 92 ± 4.0 h and C_{max} decreased from 72 ± 13.2 to 29 ± 10.3 ng/ml (Table 2). At the end of the experiments, rats were sacrificed and the implantation site was visually inspected. No traces of polymer could be detected in the tissue around the site, indicating that there had been complete erosion of the photocrosslinked poly(ester anhydride) implants.

4. Discussion

This work clearly demonstrates the suitability of photocrosslinked poly(ester anhydride)s for achieving peptide delivery such that the release rate of the peptide can be tailored by modifying the oligomer hydrophobicity of the poly(ester anhydride). These materials have potential for use in applications requiring controlled short-term release of macromolecular compounds.

The incorporation of a hydrophobic 12-carbon alkenyl chain into the oligomers decreased the erosion rate of the photocrosslinked poly(ester anhydride) implant in comparison with the implant without the alkenyl chain. This is in agreement with an earlier study done with thermoplastic poly(ester anhydride)s [7]. Since the drug release from photocrosslinked poly(ester anhydride)s is surface-erosion-controlled as discussed below, the change in the erosion rate had a direct effect on the release rate of PYY3-36. The 12-ASA-functionalization affected PYY3-36 *in vitro* release, which was incomplete even though the implant had been totally eroded. When degradation products of SAH- and 12-ASA-functionalized implants are compared, the only difference is their hydrophobicity due to 12-carbon alkenyl chain of 12-ASA-functionalized polymer (Scheme 1). Therefore, it is postulated that incomplete *in vitro* release is due to a hydrophobic interaction between PYY3-36 and hydrophobic moieties of degradation products of 12-ASA-functionalized polymer rather than an interaction between PYY3-36 and anhydride bonds. An earlier study demonstrated that *in vitro* degradation products of polyanhydride microspheres, i.e., fatty acid dimers, formed an interaction with

Table 1

In vitro release and *in vivo* release by using percent AUC method of PYY3-36 from SAH- and 12-ASA-functionalized implants (mean \pm SEM, $n = 3$ (*in vitro*) or $n = 6$ (*in vivo*)).

	Release rate (%/h)	R2 value of release	Duration of linear release (h)	Lag time of linear release (h)
SAH-functionalized (<i>in vitro</i>)	4.5 \pm 0.33	0.885 \pm 0.029	24	0
SAH-functionalized (<i>in vivo</i>)	2.0 \pm 0.10	0.984 \pm 0.002	46 \pm 2.0	22 \pm 2.0
12-ASA-functionalized (<i>in vivo</i>)	1.0 \pm 0.07	0.975 \pm 0.006	102 \pm 7.4	38 \pm 4.8

Table 2

Pharmacokinetic parameters of PYY3-36 after i.v. and s.c. solution and SAH- and 12-ASA-functionalized photocrosslinked poly(ester anhydride) implant administration to rats (mean \pm SEM, $n = 4$ for solutions and $n = 6$ for implants).

Pharmacokinetic parameters	2 μ g i.v.	20 μ g i.v.	2 μ g s.c.	20 μ g s.c.	SAH-functionalized (525 μ g)	12-ASA-functionalized (483 μ g)
C_{max} (ng/ml)	26.8 \pm 1.8 ^a	187 \pm 26.1 ^a	0.98 \pm 0.26 ^b	3.5 \pm 0.44 ^b	72.0 \pm 13.2 ^c	29.2 \pm 10.3 ^c
t_{max} (h)	0.017	0.017	0.63 \pm 0.13	0.25 \pm 0.09	42.0 \pm 4.0 ^d	92.0 \pm 4.0 ^d
$t_{1/2}$ (min)	38.8 \pm 2.3	39.8 \pm 4.1	42.3 \pm 4.2	78.0 \pm 24.0		
CL (ml/h/kg)	771 \pm 97.1	1240 \pm 110				
V_d (ml/kg)	713 \pm 81.6 ^a	1160 \pm 74.4 ^a				
AUC_{0-1ast} (h ng/ml/kg)	27.0 \pm 3.8 ^a	167 \pm 15.1 ^a	5.8 \pm 1.7	8.7 \pm 1.1	5750 \pm 972	4760 \pm 1390
$AUC_{0-\infty}$ (h ng/ml/kg)	27.6 \pm 3.7 ^a	170 \pm 15.8 ^a	6.0 \pm 1.7	10.7 \pm 1.9	5830 \pm 958	4790 \pm 1400
F% (2 μ g i.v.)			17.9 \pm 6.2	3.9 \pm 0.7 ^e	80.5 \pm 13.2 ^e	71.2 \pm 20.4 ^e
F% (20 μ g i.v.)			29.1 \pm 10.0	6.3 \pm 1.1 ^e	131 \pm 21.5 ^e	116 \pm 33.1 ^e
F% _{rel} (2 μ g s.c.)					373 \pm 61.2	330 \pm 94.5
F% _{rel} (20 μ g s.c.)					2070 \pm 340	1830 \pm 524

Statistical differences:

^a $p < 0.05$ 2 μ g i.v. vs. 20 μ g i.v.

^b $p < 0.05$ 2 μ g s.c. vs. 20 μ g s.c.

^c $p < 0.05$ SAH vs. 12-ASA.

^d $p < 0.01$ SAH vs. 12-ASA.

^e $p < 0.01$ 20 μ g s.c. vs. SAH and 12-ASA.

protein [31]. However, *in vivo* release of PYY3-36 was nearly complete from 12-ASA-functionalized poly(ester anhydride) implants, which leads to the conclusion that PYY3-36 and degradation products of 12-ASA-functionalized polymer are not interacting in the subcutaneous space.

In surface-erosion-controlled drug release, the physicochemical properties of the drug should not affect the release rate; this should be entirely controlled by polymer erosion. Earlier, propranolol HCl (M_w 296 g/mol) release from photocrosslinked poly(ester anhydride)s has been shown to be surface-erosion-controlled *in vitro* and *in vivo* (s.c. rats) [8]. In that work, *in vitro* release rates of propranolol HCl varied from 2.1% to 3.3%/h, depending on the drug loading degree (10–60%w/w), while *in vivo* release rate was 1.7%/h from the implant with a loading degree of 40% w/w. These values correspond closely to the PYY3-36 release rates *in vitro* (4.5%/h) and *in vivo* (2.0%/h) from SAH-functionalized implants.

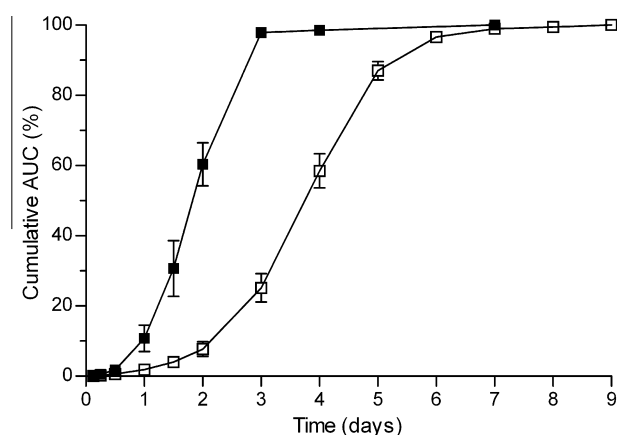


Fig. 3. *In vivo* cumulative release, by using percent AUC method, of PYY3-36 from SAH- (■) and 12-ASA-functionalized (□) photocrosslinked poly(ester anhydride) implants (mean \pm SEM, $n = 6$). The profile indicates the percent of PYY3-36 released and absorbed into plasma.

Thus, although the physicochemical properties, particularly the molecular weights of PYY3-36 (4050 g/mol) and propranolol HCl (296 g/mol), differ considerably, their release rates from SAH-functionalized implants were similar, indicating that the physicochemical properties of drugs did not influence the drug release. The tendency of *in vitro* release rates to be slightly higher than those measured *in vivo* can be explained by differences in water exposure, which is more limited in the subcutaneous space than in *in vitro* settings [32–34]. This can limit the polymer erosion based on the hydrolysis of anhydride bonds and consequently decrease the drug release rate.

Although earlier PYY3-36 has been administered subcutaneously to rodents and humans [21,35,36], its s.c. bioavailability has not been reported. In this study, the s.c. bioavailability of PYY3-36 was low (3.9–29.1%) and dose-dependent after administration as a solution, when compared with the s.c. bioavailabilities of other peptides and proteins [37,38]. However, the s.c. bioavailability of the peptide was practically 100% when it was administered in photocrosslinked poly(ester anhydride) implants. It must be noted that the administered dose of PYY3-36 was not identical in the implants (~500 μ g) and solutions (2–20 μ g) and PYY3-36 showed dose-dependent s.c. absorption based on the comparison of s.c. bioavailability and $AUC_{0-\infty}$ values of 2 and 20 μ g doses. However, the dose-dependent absorption indicated that the higher the dose, the lower was the fraction absorbed, which is opposite to the difference in bioavailability when the comparison was between solution and implant administration. The fundamental difference between solution and implant administration is the availability of peptide for absorption. After the administration of solution, PYY3-36 was immediately available for absorption but with the implant, the release rate is controlled, such that the release rate is constantly 5–10 μ g/h during the linear release phase. Based on differences in bioavailability between 2 and 20 μ g doses in solution, and between implant and solution administration, it is postulated that this low and steady release rate would be beneficial for the absorption of PYY3-36. In addition, the unreleased peptide was located in the polymer matrix where it would be protected from degradation in the subcutaneous space [4,5]. Thus, it is proposed

that s.c. administration via a biodegradable polymer represents an efficient route for sustained and controlled PYY3-36 delivery.

5. Conclusion

This work demonstrates the suitability of photocrosslinked poly(ester anhydride)s for peptide delivery since by tailoring, the hydrophobicity of poly(ester anhydride) oligomers, peptide delivery, and polymer erosion rates could be modified. PYY3-36 bioavailability was increased clearly by using photocrosslinked poly(ester anhydride)s for s.c. administration of PYY3-36 when compared with its administration in solution. Therefore, s.c. administration via a biodegradable polymer is proposed to be an efficient route for PYY3-36 delivery.

Role of the funding source

Funding sources have had no involvement in the conduct of the research or preparation of the manuscript.

Acknowledgements

Pekka Savolainen (B.Sc. Pharm.) is acknowledged for his skillful technical assistance. The financial support from the Academy of Finland (PEPBI consortium # 117906) and Graduate School in Pharmaceutical Research (J.M.), Finnish Cultural Foundation (M.K.), and Orion Farnos Research Foundation (M.K.) is acknowledged.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ejpb.2011.09.011.

References

- [1] S. Frokjaer, D.E. Otzen, Protein drug stability: a formulation challenge, *Nat. Rev. Drug Discovery* 4 (2005) 298–306.
- [2] M.R. Park, C. Chun, C.S. Cho, S.C. Song, Enhancement of sustained and controlled protein release using polyelectrolyte complex-loaded injectable and thermosensitive hydrogel, *Eur. J. Pharm. Biopharm.* 76 (2010) 179–188.
- [3] G. Schwach, N. Oudry, J.P. Giliberto, P. Broqua, M. Luck, H. Lindner, R. Gurny, Biodegradable PLGA microparticles for sustained release of a new GnRH antagonist: Part II. In vivo performance, *Eur. J. Pharm. Biopharm.* 57 (2004) 441–446.
- [4] S. Chen, J. Singh, Controlled release of growth hormone from thermosensitive triblock copolymer systems: in vitro and in vivo evaluation, *Int. J. Pharm.* 352 (2008) 58–65.
- [5] M. Katakam, W.R. Ravis, A.K. Banga, Controlled release of human growth hormone in rats following parenteral administration of poloxamer gels, *J. Controlled Release* 49 (1997) 21–26.
- [6] A. Göpferich, Mechanisms of polymer degradation and erosion, *Biomaterials* 17 (1996) 103–114.
- [7] H. Korhonen, R. Hakala, A. Helminen, J. Seppälä, Synthesis and hydrolysis behaviour of poly(ester anhydrides) from polylactone precursors containing alkenyl moieties, *Macromol. Biosci.* 6 (2006) 496–505.
- [8] J. Mönkäre, R.A. Hakala, M.A. Vlasova, A. Huotari, M. Kilpeläinen, A. Kiviniemi, V. Meretoja, K.H. Herzig, H. Korhonen, J.V. Seppälä, K. Järvinen, Biocompatible photocrosslinked poly(ester anhydride) based on functionalized poly(ϵ -caprolactone) prepolymer shows surface erosion controlled drug release in vitro and in vivo, *J. Controlled Release* 146 (2010) 349–355.
- [9] A.C. Albertsson, I.K. Varma, Recent developments in ring opening polymerization of lactones for biomedical applications, *Biomacromolecules* 4 (2003) 1466–1486.
- [10] A. Göpferich, J. Tessmar, Polyanhydride degradation and erosion, *Adv. Drug Deliv. Rev.* 54 (2002) 911–931.
- [11] R. Hakala, H. Korhonen, S. Holappa, J. Seppälä, Hydrophobicities of poly(ϵ -caprolactone) oligomers functionalized with different succinic anhydrides, *Eur. Polym. J.* 45 (2009) 557–564.
- [12] M. Neary, R. Batterham, Gut hormones: implications for the treatment of obesity, *Pharmacol. Ther.* 124 (2009) 44–56.
- [13] K. Tatamoto, V. Mutt, Isolation of 2 novel candidate hormones using a chemical method for finding naturally-occurring polypeptides, *Nature* 285 (1980) 417–418.
- [14] R. Batterham, M. Cowley, C. Small, H. Herzog, M. Cohen, C. Dakin, A. Wren, A. Brynes, M. Low, M. Ghatti, R. Cone, S. Bloom, Gut hormone PYY3-36 physiologically inhibits food intake, *Nature* 418 (2002) 650–654.
- [15] L. Degen, S. Oesch, M. Casanova, S. Graf, S. Ketterer, J. Drewe, C. Beglinger, Effect of peptide YY3-36 on food intake in humans, *Gastroenterology* 129 (2005) 1430–1436.
- [16] R.L. Batterham, M.A. Cohen, S.M. Ellis, C.W. Le Roux, D.J. Withers, G.S. Frost, M.A. Ghatti, S.R. Bloom, Inhibition of food intake in obese subjects by peptide YY3-36, *New Engl. J. Med.* 349 (2003) 941–948.
- [17] A.P. Sileno, G.C. Brandt, B.M. Spann, S.C. Quay, Lower mean weight after 14 days intravenous administration peptide YY3-36 (PYY3-36) in rabbits, *Int. J. Obesity* 30 (2005) 68–72.
- [18] P.K. Chelikani, A.C. Haver, J.R. Reeve, D.A. Keire, R.D. Reidelberger, Daily, intermittent intravenous infusion of peptide YY(3-36) reduces daily food intake and adiposity in rats, *Am. J. Physiol-Regul. Integr. Comp. Physiol.* 290 (2006) R298–R305.
- [19] I. Gantz, N. Eröndü, M. Mallick, B. Musser, R. Krishna, W.K. Tanaka, K. Snyder, C. Stevens, M.A. Stroh, H.Y. Zhu, J.A. Wagner, D.J. MacNeil, S.B. Heymsfield, J.M. Amatruda, Efficacy and safety of intranasal peptide YY3-36 for weight reduction in obese adults, *J. Clin. Endocrinol. Metab.* 92 (2007) 1754–1757.
- [20] R. Steinert, B. Poller, C. Castelli, J. Drewe, C. Beglinger, Oral administration of glucagon-like peptide 1 or peptide YY 3-36 affects food intake in healthy male subjects, *Am. J. Clin. Nutr.* 92 (2010) 810–817.
- [21] B. Sloth, L. Davidsen, J. Holst, A. Astrup, Effect of subcutaneous injections of PYY1-36 and PYY3-36 on appetite, ad libitum energy intake, and plasma free fatty acid concentration in obese males, *Am. J. Physiol-Endocrinol. Metab.* 293 (2007) E604–E609.
- [22] A. Helminen, H. Korhonen, J. Seppälä, Crosslinked poly(ester anhydride)s based on poly(ϵ -caprolactone) and polylactide oligomers, *J. Polym. Sci. Polym. Chem.* 41 (2003) 3788–3797.
- [23] J.A. Burdick, A.J. Peterson, K.S. Anseth, Conversion and temperature profiles during the photoinitiated polymerization of thick orthopaedic biomaterials, *Biomaterials* 22 (2001) 1779–1786.
- [24] D. Chu, X. Fu, W. Liu, K. Liu, Y. Li, Pharmacokinetics and in vitro and in vivo correlation of huperzine A loaded poly(lactic-co-glycolic acid) microspheres in dogs, *Int. J. Pharm.* 325 (2006) 116–123.
- [25] S. Siegel, N.J. Castellan Jr., The case of k independent samples, in: J.D. Anker (Ed.), *Nonparametric Statistics for the Behavioral Sciences*, second ed., McGraw-Hill Inc., New York, 1988, pp. 190–223.
- [26] A. Göpferich, L. Schedl, R. Langer, The precipitation of monomers during the erosion of a class of polyanhydrides, *Polymer* 37 (1996) 3861–3869.
- [27] M.J. Kipper, B. Narasimhan, Molecular description of erosion phenomena in biodegradable polymers, *Macromolecules* 38 (2005) 1989–1999.
- [28] N. Nonaka, S. Shioda, M. Niehoff, W. Banks, Characterization of blood-brain barrier permeability to PYY3-36 in the mouse, *J. Pharmacol. Exp. Ther.* 306 (2003) 948–953.
- [29] C. Beglinger, B. Poller, E. Arbit, C. Ganzoni, S. Gass, I. Gomez-Orellana, J. Drewe, Pharmacokinetics and pharmacodynamic effects of oral GLP-1 and PYY3-36: a proof-of-concept study in healthy subjects, *Clin. Pharmacol. Ther.* 84 (2008) 468–474.
- [30] H.R. Constantino, M.S. Kleppe, A. Stoudt Cohen, A.P. Sileno, Intranasal PYY formulations with improved transmucosal pharmacokinetics, US Patent Application No. US 2007/0232537 A1, 2007.
- [31] Y. Tabata, S. Gutta, R. Langer, Controlled delivery of systems for proteins using polyanhydride microspheres, *Pharm. Res.* 10 (1993) 487–496.
- [32] K. Mäder, Y. Crémilleux, A. Domb, J. Dunn, H. Swartz, In vitro/in vivo comparison of drug release and polymer erosion from biodegradable P(FAD-SA) polyanhydrides—a noninvasive approach by the combined use of electron paramagnetic resonance spectroscopy and nuclear magnetic resonance imaging, *Pharm. Res.* 14 (1997) 820–826.
- [33] M. Changez, V. Koul, B. Krishna, A.K. Dinda, V. Choudhary, Studies on biodegradation and release of gentamicin sulphate from interpenetrating network hydrogels based on poly(acrylic acid) and gelatin: in vitro and in vivo, *Biomaterials* 25 (2004) 139–146.
- [34] M. Pulkkinen, M. Malin, J. Bohm, T. Tarvainen, T. Wirth, J. Seppälä, K. Järvinen, In vivo implantation of 2,2'-bis(oxazoline)-linked poly- ϵ -caprolactone: proof for enzyme sensitive surface erosion and biocompatibility, *Eur. J. Pharm. Sci.* 36 (2009) 310–319.
- [35] R.A. Pittner, C.X. Moore, S.P. Bhavsar, B.R. Gedulin, P.A. Smith, C.M. Jodka, D.G. Parkes, J.R. Paterniti, V.P. Srivastava, A.A. Young, Effects of PYY[3-36] in rodent models of diabetes and obesity, *Int. J. Obes. Relat. Metab. Disord.* 28 (2004) 963–971.
- [36] R. Moriya, S. Mashiko, A. Ishihara, T. Takahashi, T. Murai, J. Ito, Y. Mitobe, Z. Oda, H. Iwasa, F. Takehiro, A. Kanatani, Comparison of independent and combined chronic anti-obese effects of NPY Y2 receptor agonist, PYY(3-36), and NPY Y5 receptor antagonist in diet-induced obese mice, *Peptides* 30 (2009) 1318–1322.
- [37] C.J.H. Porter, S.A. Charman, Lymphatic transport of proteins after subcutaneous administration, *J. Pharm. Sci.* 89 (2000) 297–310.
- [38] M. Rowland, T.N. Tozer, *Clinical Pharmacokinetics: Concepts and Applications*, third ed., Lippincott Williams & Wilkinson, Philadelphia, 1995.