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Research paper

In vivo pharmacokinetics of celecoxib loaded endcapped PCLA-PEG-PCLA thermogels in rats after subcutaneous administration

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ABSTRACT

Injectable thermogels based on poly(ε-caprolactone-co-lactide)-b-poly(ethylene glycol)-b-poly(ε-caprolactone-co-lactide) (PCLA-PEG-PCLA) containing an acetyl- or propyl endcap and loaded with celecoxib were developed for local drug release. The aim of this study was to determine the effects of the composition of the celecoxib/PCLA-PEG-PCLA formulation on their *in vivo* drug release characteristics. Furthermore, we want to obtain insight into the *in vitro-in vivo* correlation. Different formulations were injected subcutaneously in rats and blood samples were taken for a period of 8 weeks. Celecoxib half-life in blood increased from 5 h for the bolus injection of celecoxib to more than 10 days for the slowest releasing gel formulation. Sustained release of celecoxib was obtained for at least 8 weeks after subcutaneous administration. The release period was prolonged from 3 to 6–8 weeks by increasing the injected volume from 100 to 500 µL, which also led to higher serum concentrations in time. Propyl endcapping of the polymer also led to a prolonged release compared to the acetyl endcapped polymer (49 versus 21 days) and at equal injected dose of the drug in lower serum concentrations. Increasing the celecoxib loading from 10 mg/mL to 50 mg/mL surprisingly led to prolonged release (28 versus 56 days) as well as higher serum concentrations per time point, even when corrected for the higher dose applied. The *in vivo* release was about twice as fast compared to the *in vitro* release for all formulations. Imaging of organs of mice, harvested 15 weeks after subcutaneous injection with polymer solution loaded with infrared-780 labelled dye showed no accumulation in any of these harvested organs except for traces in the kidneys, indicating renal clearance. Due to its simplicity and versatility, this drug delivery system has great potential for designing an injectable to locally treat osteoarthritis, and to enable tuning the gel to meet patient-specific needs.

1. Introduction

Celecoxib is a non-steroidal anti-inflammatory drug (NSAID) and a selective inhibitor of *cyclo*-oxygenase-2 (COX-2) [1]. Celecoxib is taken orally once or twice a day in pain management, for instance, by patients with osteoarthritis [2]. To reach local therapeutic concentrations, high daily dosing is necessary due to the low oral availability and the fact that only a small portion of the administered dose reaches the inflamed joints. Celecoxib is 97% protein bound, with a large apparent volume of distribution (> 1 L/kg), suggesting extensive distribution into tissues [2,3]. High systemic concentrations are unwanted since concerns have risen about the toxicity of celecoxib, for instance in myocardial function [4]. The best option to decrease the risk of systemic side-effects is through local administration of the drug in the target tissue. Direct intra-articular injection of a drug in patients with osteoarthritis is not desirable due to rapid intra-articular drug wash-out combined with the fact that repeated intra-articular injections are not patient friendly and pose a potential risk of infection [5]. Therefore, research has been focused on the development of injectable drug delivery systems based on hydrogels with a well-controlled and sustained release [6–14].

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Table 1
Description of the different formulations used for *in vivo* studies.

Formulation	Endcapping	Injected volume (μL)	Celecoxib concentration (mg/mL)	Celecoxib dose (mg)	Polymer concentration in PBS (% w/w)
A	Acetyl	500	10	5	25
B	Acetyl	100	50	5	20
C	Acetyl	500	50	25	20
D	Acetyl	500	10	5	20
E	Propyl	500	10	5	20

We have previously shown in a relevant animal model that acetyl endcapped poly(ε-caprolactone-co-lactide)-poly(ethylene glycol)-poly (ε-caprolactone-co-lactide) (PCLA-PEG-PCLA) based thermoreversible hydrogels have excellent potential for the local release of celecoxib with sustained *in vivo* release kinetics of 4–8 weeks. In addition, good biocompatibility after both subcutaneous and intra-articular administration was observed [15,16]. Recently, intradiscal injections of PCLA-PEG-PCLA hydrogels loaded with celecoxib were performed in ten client-owned dogs with chronic low back pain, of which 9 out of 10 dogs showed clinical improvement [17].

These PCLA-PEG-PCLA hydrogels are very interesting for different applications, since release kinetics of these polymer systems are likely tunable by changing multiple factors. Firstly, the volume of injected gel and thus the therapeutic dose can be altered. Secondly, the polymer concentration in the formulation can be changed, which leads to variation in network density of the gel, thereby influencing the release kinetics. Thirdly, the capping group of hydroxyl ends of the tri-block copolymer can be altered, resulting in different release characteristics. As a last factor, the amount of the drug in the formulation can be changed. Indeed, in a previous study we demonstrated that increasing the celecoxib concentration resulted in longer *in vitro* releases [15]. The choosen animal model was the rat model, due to the extensive information available of oral administration of celecoxib in rats, and to be able to compare the obtained results with other studies [18,19].

The primary aim of this study was to determine the effects of the injection volume of the formulation, polymer concentration and capping group of celecoxib/PCLA-PEG-PCLA formulations on the *in vivo* drug release. The second aim of the present study was to get insight into the relation between the *in vitro* and *in vivo* release characteristics of the formulations.

2. Materials and methods

2.1. Materials

Celecoxib was obtained from LC Laboratories, USA. All other chemicals were obtained from Sigma Aldrich.

2.2. Synthesis of acetyl- and propyl endcapped PCLA-PEG-PCLA

The acetyl- and propyl endcapped PCLA-PEG-PCLA triblock copolymers used in this study were synthesized and characterized as described previously [20]. In short, a three-neck round-bottom flask equipped with a Dean Stark trap and a condenser was used. PEG_{1500}, L-lactide, ε-caprolactone and toluene were introduced and, while stirring, heated to reflux (~140 °C) under a nitrogen atmosphere. The Dean stark apparatus was used in azeotropic drying by distillation of toluene/water (ca. 50% volume of the initial volume). Next, the solution was cooled down to < 80 °C and tin(II) 2-ethylhexanoate was added. Ring-opening polymerization was carried out at 110–120 °C overnight under a nitrogen atmosphere. The solution was cooled down to room temperature and dichloromethane and triethylamine were added. Subsequently, the solution was cooled to 0 °C in an ice bath, and while stirring, an excess of acetyl chloride or propyl chloride (depending on the required endcap) was added dropwise and acylation/propylation was allowed to proceed for three hours. Next, dichloromethane was removed under vacuum at 60–65 °C, ethyl acetate was subsequently added and triethylamine hydrochloride salts were removed by filtration. The polymer was precipitated by adding a 1:1 mixture of pentane and diethyl ether. Upon storage at −20 °C, the polymer separated as a waxy solid from which non-solvents containing unreacted monomers and the excess of acyl chloride could be decanted easily. The precipitated polymer was dried under vacuum and obtained in yield of 85%. The polymer was characterized by ^{1}H NMR and GPC, as described previously [21].

2.3. Preparation of PCLA-PEG-PCLA celecoxib formulations

The formulations were prepared by mixing 5 g of PCLA-PEG-PCLA with 20 mL PBS buffer (43 mM Na_2HPO_4, 9 mM NaH_2PO_4, 75 mM NaCl; pH 7.4, 280 mOsm/kg) (20% formulation) or 5 g of PCLA-PEG-PCLA with 15 mL PBS buffer (25%). Celecoxib was added to these formulations at a final concentration of 10 or 50 mg/mL. Since autoclaving has no negative effect on the formulations, all formulations were autoclaved for 15 min at 121 °C [16]. After cooling down to approximately 40 °C, the mixtures were vortexed for 2 min and subsequently incubated at 4 °C for 48 h to allow formation of homogeneous polymer solutions. Rheological analysis of 300 μL of the polymer solutions was determined as described previously [15]. An overview of the formulations prepared for this study is given in Table 1.

2.4. In vivo celecoxib release

The Animal Ethics Committee of the Erasmus Medical Center, Rotterdam, The Netherlands, approved all conducted procedures (agreement number EMC2255(116-11-02)). It was previously shown that there is a gender difference in the pharmacokinetics of celecoxib in rats, with celecoxib being eliminated from the plasma 4-times faster in males compared to females [18]. Only male rats were used in our study. Fourteen-week-old (400–450 g) male Wistar rats (Charles River Nederland BV, Maastricht, The Netherlands) were housed in the animal facility of the Erasmus Medical Center, with a 12-h light-dark regime, at 21 °C. Animals were fed standard food pellets and water *ad libitum*. Experiments started after an acclimatization period of 2 weeks. To investigate the pharmacokinetics of the different PCLA-PEG-PCLA/celecoxib formulations, rats (6 animals per group (5 groups in total)) were injected subcutaneously in the neck region with aseptically prepared and autoclaved PCLA-PEG-PCLA formulations as described in Table 1.

As a control for absolute bioavailability and determination of the elimination half-life of celecoxib, nine rats received an intravenous bolus injection of 200 μL celecoxib. Since the aqueous solubility of celecoxib is limited, it was dissolved in polyethylene glycol (PEG) 400:water in a 2:1 ratio (w/v) in a concentration of 10 mg/mL as described before by Paulson *et al* [18].

At predetermined time points between 0 and 56 days, blood samples (500 μL) were taken from the lateral tail vein and collected in Vacutainer SST™ II Advance (BD Plymouth) tubes that contained silica (clot activator). After spinning down the cells (3500 rpm, 10 min), celecoxib was extracted from the serum using ethyl acetate [16]. In total, 100 μL serum was mixed with 100 μL internal standard (200 ng/mL parecoxib in 5% BSA). Then, 200 μL 0.1 M sodium acetate buffer (pH 5.0) was added, followed by ethyl acetate (1 mL) and the samples

were vortexed for 10 min. Subsequently, samples were centrifuged at 11,000 rpm for 10 min and stored at −80 °C for 30 min. The upper ethyl acetate phase was transferred into HPLC glass vials and evaporated under nitrogen atmosphere. Next, the residues were dissolved in 100 µL of methanol and celecoxib concentration in the samples was analyzed by LC-MS. Per sample, 5 µL was injected onto a Kinetex® C18 (30 * 3.0 mm, particle size of 2.6 µm) analytical column (Phenomenex, Utrecht, NL). Separation was performed at a flow rate of 500 µL/min, with a total run time of 3 min. The mobile phases consisted of acetonitrile/water (1/1 v/v) (A), and acetonitrile/methanol (1/1 v/v) (B). Samples were separated using the following gradient A/B v/v: 0–0.6 min, 100/0; 0.6–0.7 min, 100/0–30/70; 0.7–1.6 min, 30/70–0/100; 1.6–2.4 min, 0/100; 2.4–2.7 min, 0/100–100/0; 2.7–3.0 min, 100/0. Column temperature was set at 40 °C. The column effluent was introduced by an atmospheric pressure chemical ionization (APCI) interface (Sciex, Toronto, ON) into an API3000 mass spectrometer. For maximal sensitivity, the mass spectrometer was operated in negative ion multiple-reaction monitoring (MRM) mode. Peaks were identified by comparison of retention time and mass spectra of standards. For each component two ion transitions were monitored, celecoxib: 380.3 → 316.3 and 380.3 → 276.3 (collision energy: −50 V), and parecoxib: 369.3 → 250.2 and 369.3 → 234.2 (collision energy: −30 V). The following MS parameters were used: nebulizer gas: 10 psi; curtain gas: 10 psi; ion current: −2 µA; source temperature: 500 °C; gas flow 1: 30 psi; gas flow 2: 20 psi: decluster potential: −70 V and entrance potential: −10 V.

2.5. Data analysis

LC-MS data were analyzed with Analyst software version 1.4.2 (Applied Biosystems, Nieuwerkerk a/d IJssel, The Netherlands). Celecoxib peak areas were corrected for the parecoxib recovery, and concentrations were calculated using celecoxib standards prepared in rat serum ranging from 0.5 to 1000 ng/mL. The calibration curve was linear in this range (r = 0.9997). Single blood sample data were used to construct the plasma level curves.

The pharmacokinetic characterization of celecoxib was analyzed using PK Solver, Version 2.0, an add-in program for pharmacokinetic and pharmacodynamic data analysis in Microsoft Excel [22]. Non-compartmental modeling was carried out according to conventional pharmacokinetic principles. The fraction released *in vivo* was determined by dividing the total area under the curve (AUC) at different time points by the $AUC_{0-\infty}$.

2.6. In vitro celecoxib release

Formulations that were subcutaneously injected in rats were also studied for their *in vitro* release characteristics. In total, 100 or 500 µL formulations were transferred into cell culture tubes (16 * 100 mm) using a syringe. The tubes were incubated for 15 min at 37 °C to allow gel formation. Next, 5 mL PBS buffer (43 mM Na_2HPO_4, 9 mM NaH_2PO_4, 75 mM NaCl; pH 7.4, 280 mOsm/kg) with 0.2% w/w Tween® 80 for the lower dose and 1% w/w Tween® 80 for the higher dose was added, and formulations were shaken at 300 RPM. Tween® 80 was added to solubilize the released celecoxib and to maintain sink conditions. At predetermined time points, samples of 2.5 mL were withdrawn and 2.5 mL fresh PBS was added. The celecoxib concentration in the different release samples was determined by LC-UV as described in the Supporting Information.

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.ejpb.2018.07.026.

2.7. In vivo imaging in mice

All experimental procedures for *in vivo* imaging were approved by the Subcommittee on Research Animal Care at Leiden University Medical Center. Male FVB mice (4–8 weeks of age, from the LUMC breeding facility) were used for the experiment. Two mice were injected subcutaneously with 10 µL of a solution containing 20% acetyl end-capped polymer solution loaded with a near-infrared dye (IR-780 iodide). IR-780 iodide was chosen due to its poor aqueous solubility, like celecoxib. The total dose of near-infrared dye was 0.05 µg. Fifteen weeks after subcutaneous injection, the mice were sacrificed, dissected, and *ex vivo* scans of the site of injection and all major organs were made to check the redistribution of the dye to the rest of the body.

Fluorescence imaging was performed with an IVIS spectrum animal imaging system (Perkin Elmer/Caliper LifeSciences, Hopkinton, MA). For spectral unmixing, an image cube was collected on the IVIS Spectrum with 18 narrow band emission filters (20 nm bandwidth) that assist in significantly reducing autofluorescence by the spectral scanning of filters and the use of spectral unmixing algorithms. Fluorescence regions were identified and spectrally unmixed using Living Image 4.3.1 software.

3. Results and discussion

3.1. In vivo pharmacokinetics of a single IV bolus injection (4 mg/kg celecoxib)

Intravenous (IV) administration of a single dose of 4 mg/kg celecoxib was performed as described previously by Paulson et al. [18]. A 200 µL solution of polyethylene glycol 400/saline (2:1, v/v) containing 10 mg/mL CLB was injected to obtain reference serum samples with different celecoxib concentrations over time and to enable calculation of the relative bioavailability for the SC administered gel formulations. The serum concentration after IV administration is shown in Fig. 1.

By definition, the first sampling point after IV administration is the C_{max} (10 min after administration), followed by a rapid decline of the serum drug concentration. Twenty-four hours post-injection, the drug concentration was only 0.1% of the C_{max}. Fig. 1 shows that the celecoxib serum concentration versus time plot can be divided in a distribution phase (first two hours) followed by an elimination phase (2–24 h). The $t_{1/2}$-values of the distribution phase (α) and the elimination phase (β) were 0.1 and 4.7 h respectively, which is in line with the results of Paulsen *et al.* ($t_{1/2}$ = 3.7 h) [18]. The calculated pharmacokinetic values are given in Table 2. The clearance of the drug was 8.6 mL/min/kg (dose/$AUC_{0-\infty}$; 2,000,000 ng/7753 ng/mL * h = 258 mL/hr [=8.6 mL/min/kg]). This value is very close to what was found earlier for clearance of celecoxib in male rats (7.8 mL/min/kg [18]). Furthermore the $AUC_{0-\infty}$ of our reference group (7.8 µg/mL * h) is the same as that found by Paulson et al., 8.6 µg/mL * h (2.15 µg/ml * h for a total dose of 1 mg/kg, while our dose was 4 mg/kg; 4 * 2.15 = 8.6 µg/mL * h). Since our results are very similar to previous pharmacokinetic values obtained in a different study [18], we

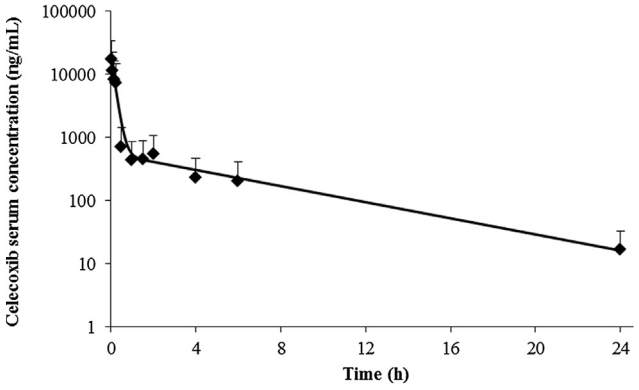

Fig. 1. Celecoxib serum concentrations measured after intravenous injection of 200 µL of 10 mg/mL celecoxib in PEG400/water solution (n = 3 per time point, mean ± SEM).

Table 2
Pharmacokinetic parameters of celecoxib after a single IV administration (2.0 mg dose, 4 mg/kg) in rats.

Parameter	Unit	Values
Dose	mg	2.0
$t_{1/2}$ Alpha	h	0.1
$t_{1/2}$ Beta	h	4.7
$AUC_{0-\infty}$	ng/mL * h	7753

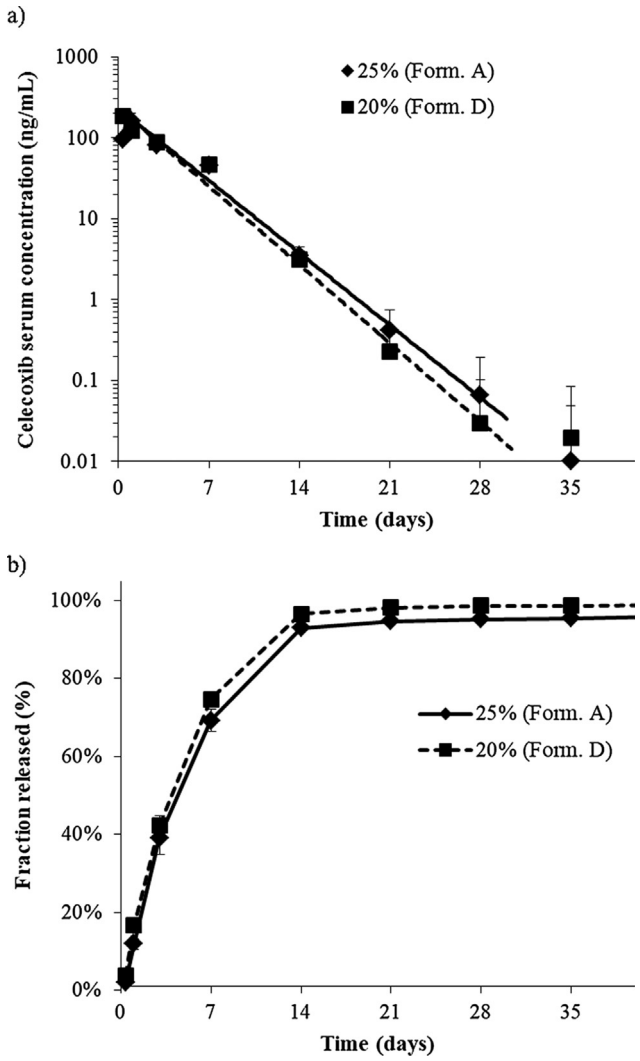

Fig. 2. Effect of polymer concentration of the formulation on *in vivo* celecoxib release; (a) the celecoxib serum concentrations after subcutaneous injection of Formulation A and D (mean ± SEM, n = 6) and (b) cumulative fraction absorbed/released from Formulation A and D.

consider these results consistent and therefore they were used to evaluate the *in vivo* kinetics of the different slow release formulations of Table 1.

3.2. Effect of polymer concentration of the hydrogel formulation on celecoxib release

Fig. 2 shows the serum concentrations of celecoxib after injection of formulation A and D (Table 1). The difference between the two formulations is the polymer concentration (25% versus 20%, respectively). C_{max} (150–190 ng/mL) was reached after 1 day, followed by a gradual drop of celecoxib concentration during the next 28 days (from 190 to 0.06 ng/mL). These results show that the injected formulations released

Table 3
Pharmacokinetic parameters of celecoxib after subcutaneous administration of different polymer formulations containing celecoxib in rats.

		Formulation				
Parameter		A	B	C	D	E
Dose	mg	5	5	25	5	5
$AUC_{0-\infty}$	ng/mL * d	778	811	2508	800	792
$t_{1/2}$	days	4.1	7.3	10.6	4.0	6.9
Cmax	ng/mL	159	205	695	186	94
F	%	96%	100%	64%	99%	98%

AUC = Area under the curve; $t_{1/2}$ = apparent half-life; Cmax = maximum serum concentration; F = systemic bioavailability.

the loaded celecoxib during at least 28 days. The results of Figs. 1 and 2 were used to calculate the bioavailability of celecoxib after SC administration [23]:

$$\text{Bioavailability} = (AUC_{sc} * Dose_{iv})/(AUC_{iv} * Dose_{sc})$$

It appears that the bioavailability for formulation A and D was 96% and 99% respectively, which demonstrated that the full dose of formulated celecoxib was released and reached the bloodstream during the 4-week period. Half-life ($t_{1/2}$) for both formulation A and D was calculated using PK Solver according to conventional pharmacokinetic principles [22]. Since the rate of decline of the celecoxib serum concentration is not due to elimination alone, but also due to other factors, such as absorption rate and/or distribution rate, the observed half-life is called apparent half-life. An apparent half-life of 4 days (Table 3) was obtained and a sustained release of 28 days was reached for both formulations. No significant differences were observed between the two formulations.

Rheological analysis showed that the storage modulus of the 25% thermogel (220 Pa at 40 °C) was higher than that of the 20% thermogel (166 Pa at 40 °C). Therefore, it was anticipated that longer release would be obtained for the 25% polymer formulation compared to the 20% one. However, no differences in the *in vivo* pharmacokinetics were observed. The release of drugs from gels is dependent on the diffusion of the drug through the gel and erosion of the gel. Obviously, the differences in erosion and diffusion between the 20 and 25% gels are not that large.

3.3. Effect of celecoxib loading of the hydrogel formulation on celecoxib release

The effect of celecoxib loading on the release was determined by comparing a 20% gel containing 10 mg/mL celecoxib (formulation D) with the same gel containing a five-time higher loading (50 mg/mL, formulation C). The injection volume was the same for both formulations. Celecoxib serum concentration after injection of the gel with 10 mg/mL celecoxib led to a C_{max} of 186 ± 64 ng/mL 8 h post-injection, while the observed C_{max} after administration of the gel with 50 mg/mL celecoxib was 695 ± 322 ng/mL. This means that the C_{max} indeed scales, within the experimental error, with the administered dose (Table 3). In case of the 50 mg/mL loading, celecoxib concentrations dropped to 278 ± 103 ng/mL after 24 h and from day 3, a continuous and sustained drug release was observed with average serum concentrations between 80 and 5 ng/mL 4–8 weeks after injection. At week 7 as well as at week 8, 3 out of the 6 animals still showed measurable celecoxib serum concentrations, respectively between 9 and 18 and 6–16 ng/mL. The fact that there is still a fair amount of celecoxib measurable in the serum at the end-point (8 weeks) indicates that a full drug release from the gel was not achieved at this point in time yet.

For formulation C, an initial peak in the 24 h accounted for 17% and the first 3 days for 30% of the total release, after that the dose was released in a sustained mode over the course of 4–8 weeks (Fig. 3). For

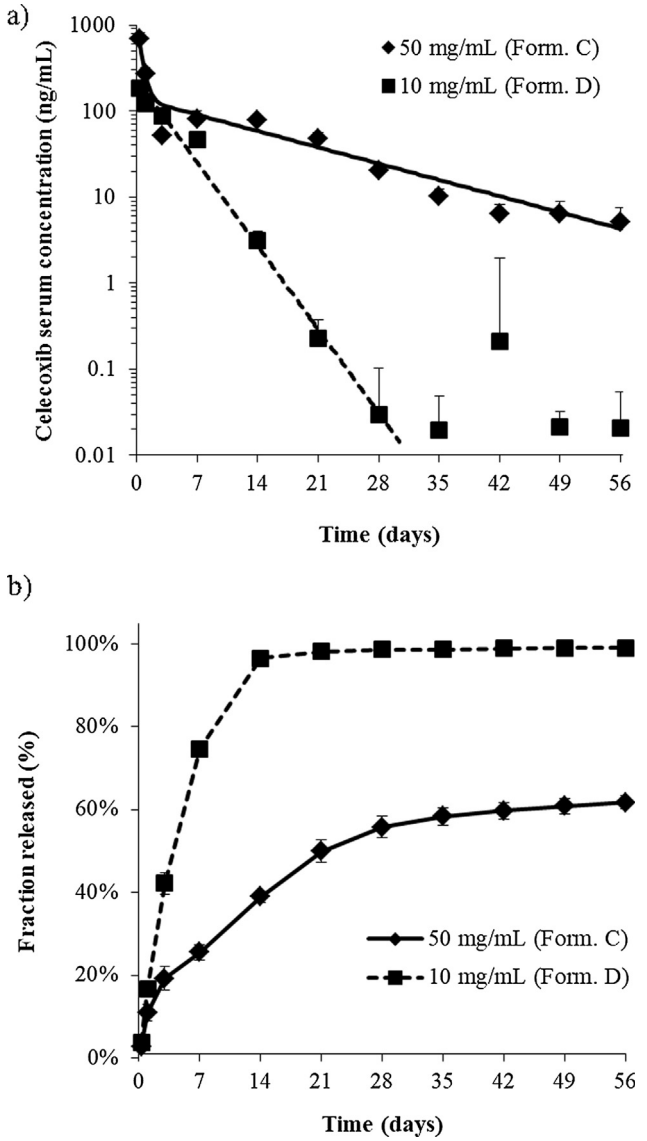

Fig. 3. Effect of celecoxib loading of the formulation (50 mg/mL versus 10 mg/mL) on celecoxib release; (a) the celecoxib serum concentrations after subcutaneous injection of Formulation C and D (mean ± SEM, n = 6) and (b) cumulative fraction absorbed/released from Formulation C and D.

formulation D, the initial release after 24 h was 17% and the release in the first 3 days accounted for 43% of the total release, the remaining 60% was released in a sustained mode over 1–4 weeks.

The bioavailability was 99% for formulation D while this was only 64% for formulation C. This means that for formulation C, there is 36% celecoxib "missing". There is no reason to believe that other kinetic properties play a role or that the rest of the celecoxib was excreted in a different way (lymphatic system) without being measured in the bloodstream [24,25]. Therefore, it is highly likely that this amount is still present subcutaneously and will be released after the 8-week period.

The sustained release period was greatly prolonged by increasing the celecoxib loading in the formulation. The formulation containing 10 mg/mL celecoxib showed a 3 week (21 days) release profile, whereas the formulation containing 50 mg/mL celecoxib showed more than 8 weeks (56 days) release. Due to the higher dose (50 mg/mL and 10 mg/mL represent a total dose of 25 mg versus 5 mg respectively), the serum concentrations were higher, but after 2 weeks the difference in serum concentration was more than 5-times higher, which cannot be explained solely by the higher drug loading. This phenomenon might be explained by celecoxib-polymer interactions.

We have shown previously that with increasing celecoxib loading of the gels, the dissolution time of the gels increased and thus concluded that the celecoxib loading has an effect on the stability of the gel through the hydrophobicity of the celecoxib and its interaction with PCLA-PEG-PCLA [15]. Furthermore, the high amount of encapsulated celecoxib led to the presence of celecoxib in a partly crystallized state within formulation C (50 mg/mL), which was not the case for formulation D (10 mg/mL). At a concentration of 10 mg/mL the celecoxib is fully dissolved, and at a celecoxib concentration of 50 mg/mL needle shaped crystals were observed (see Fig. 4) [16]. It takes time before the crystals dissolve, and therefore the formulation with the fully dissolved drug will release its content faster than the formulation with celecoxib in a partly crystallized state [25].

3.4. Effect of injected volume

Formulation B and C, which are identical (same polymer, same polymer concentration, and celecoxib concentration) except for the injected volume (100 μL versus 500 μL), were compared for their *in vivo* release characteristics (see Fig. 5). The injected dose of celecoxib was therefore 5-times higher for formulation C than for formulation B. Due to this dose difference, the C_{max} was much higher for formulation C than for B (695 and 206 ng/mL, respectively). The initial release of celecoxib is coming from the outer surface of the depot. As seen by us and by others, hydrogels form a spherical shape when injected subcutaneously [26,27]. A spherical 500 μL depot has an outer surface of approximately 300 mm^2 (radius is ~5 mm) compared to 100 mm^2 for a 100 μL depot (radius is ~3 mm). This means that the absolute amount of celecoxib at the outer surface of a 500 μL depot is approximately 3-times higher as compared to a 100 μL depot. This 3-fold difference is also observed in the C_{max}, of formulation C compared to

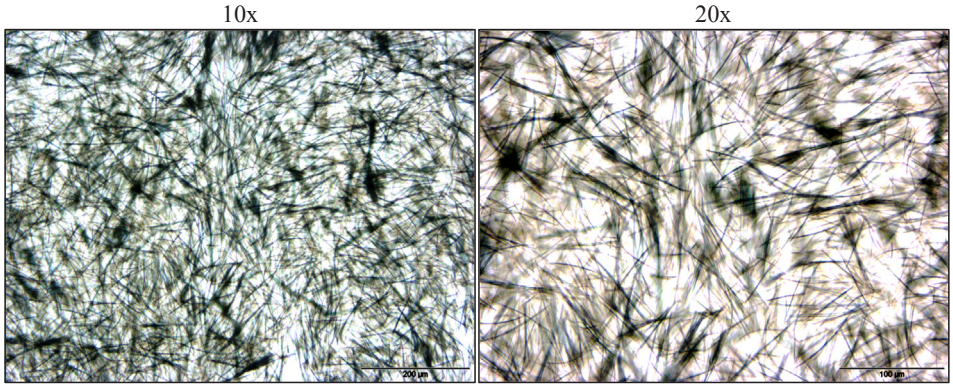

Fig. 4. Microscopic evaluation of formulation C, 20% acetyl endcapped polymer in PBS containing 50 mg/mL celecoxib (magnification 10× and 20×).

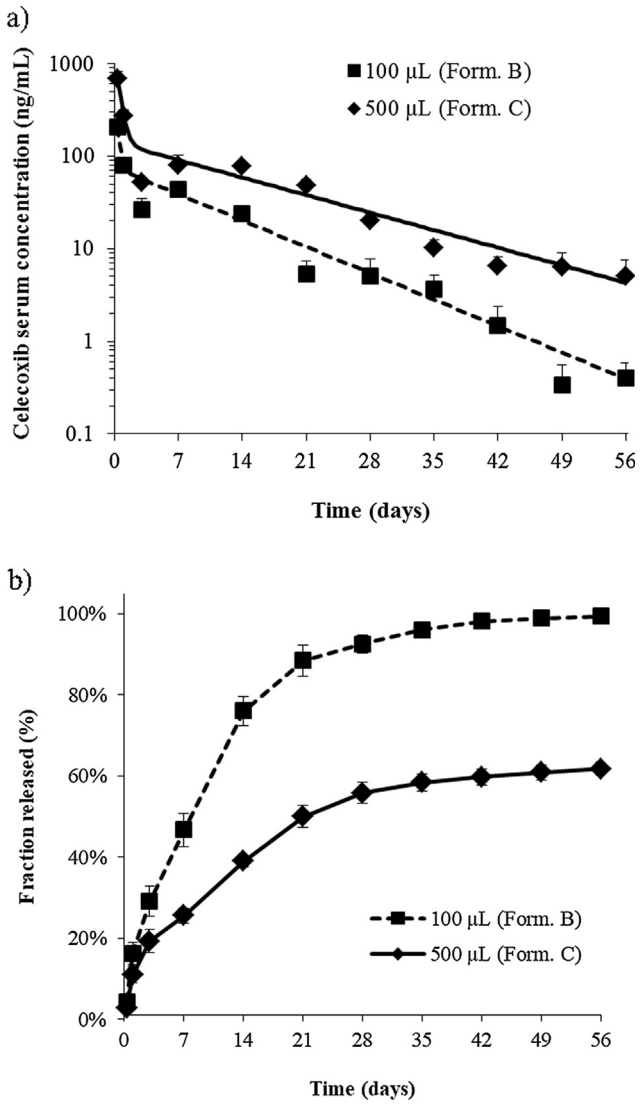

Fig. 5. Effect of injection volume on celecoxib release; (a) the celecoxib serum concentrations after subcutaneous injection of Formulation B and C (mean ± SEM, n = 6) and (b) cumulative fraction absorbed/released from Formulation B and C.

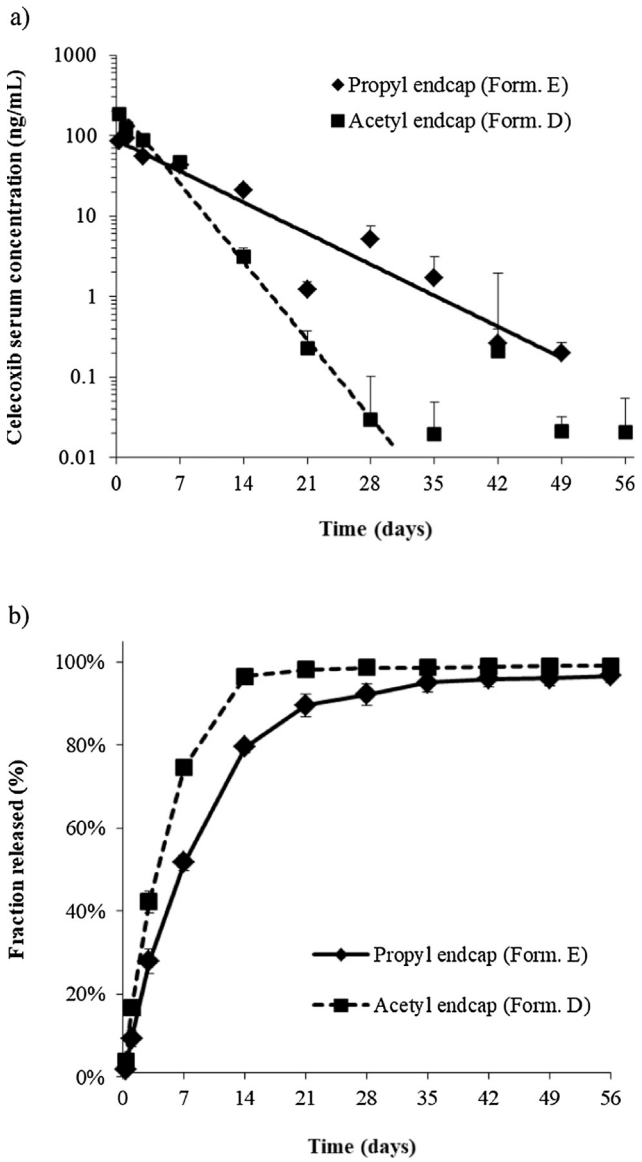

Fig. 6. Effect of polymer type on celecoxib release; (a) the celecoxib serum concentrations after subcutaneous injection of Formulation D and E (mean ± SEM, n = 6), and (b) cumulative fraction absorbed/released from Formulation D and E.

formulation B (see Table 3).

The apparent serum half-life was prolonged from 7 to 11 days when injecting a 500 µL depot instead of 100 µL. In case of formulation B, the celecoxib concentrations dropped from 205 ng/mL to an average of 27 ± 20 ng/mL after 3 days, followed by a continuous and sustained release with average serum concentrations between 4 and 25 ng/mL for 2–6 weeks. For Formulation B, a relative bioavailability of 100% was calculated showing that this composition led to a complete release of the encapsulated celecoxib.

Changing the injected volume did not only have an effect on the serum concentrations per time-point as described above, but also had a direct effect on the release period from the depot; the 500 µL formulation C led to a release period of 8 weeks, while injection of 100 µL formulation B led to a sustained release of 3–6 weeks. This difference can also be explained by the larger volume of the depot, since it takes longer for the larger depot to fully degrade. Consequently, the celecoxib release from the larger depot is prolonged compared to the smaller depot.

As stated before (Section 3.3), the bioavailability of formulation C is only 64% and it is highly likely that the remaining 36% was still present subcutaneously at the 8-week time-point at which the *in vivo* experiments ended. This indicates that the actual release period from formulation C is probably longer than 8 weeks.

3.5. Effect of polymer type

To determine the effect of a different endcap on the *in vivo* release kinetics of celecoxib, a formulation was tested where the acetyl-endcap of the PCLA-PEG-PCLA triblock copolymer (Formulation D) was replaced by a propyl group (Formulation E). This propyl group theoretically results in stronger hydrophobic interactions and thereby forming a stronger gel when the temperature is above the gelling temperature (> 28 °C).

Both formulations (D and E) showed a ~100% relative bioavailability, whereas the C_{max} after administration of formulation E was significantly lower compared to formulation D (94 versus 186 ng/mL (Student's *t*-test, p = 0.01)). In addition to a lower initial release for formulation E, also a more sustained release after 3 days was obtained compared to formulation D (Fig. 6). After 4 weeks, the serum concentration for formulation E was 5 ± 2 ng/mL while this was almost

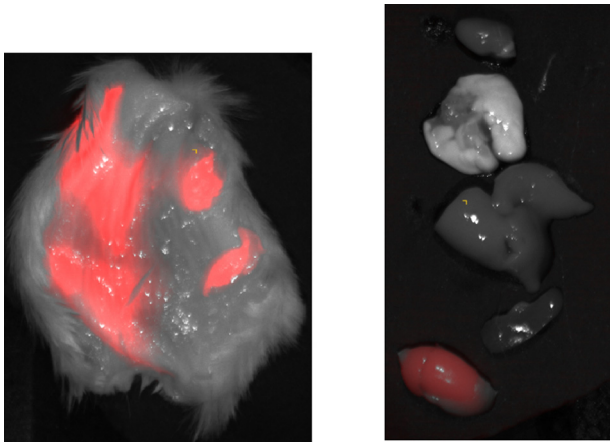

Fig. 7. (a) IVIS images of the site of injection and (b) the harvested organs 15 weeks after subcutaneous injection.

zero (0.1 ± 0.1 ng/ml) for formulation D. Finally, the propyl encapped polymer showed a sustained release up to 49 days, whereas the acetyl endcapped polymer had a release of only 21 days. These data all points to a stronger gel for formulation E than for D, which can likely be ascribed to the more hydrophobic propyl capping groups which in turn slows down the release of the loaded celecoxib.

Interestingly, at day 21, a celecoxib concentration of 1.2 ng/mL was determined for formulation E in all six rats, whereas this concentration increased to 5.2 ng/mL at day 28. Since the lower concentration at day 21 was determined in all six rats it is unlikely due to an analytical artifact. A plausible explanation of this phenomenon is that there is a biphasic drug release. Up to 21 days, the celecoxib release is diffusion driven, but after these 3 weeks polymer degradation starts to occur, leading to the second phase of celecoxib release [28].

3.6. In vitro-in vivo correlation (IVIVC)

The *in vitro* release characteristics of the formulations of Table 1 are shown in the Supporting Information. The drug release *in vitro* followed the same trends as observed *in vivo*. No differences in release were observed between the formulation with 20% w/w polymer (A) versus 25% w/w polymer (B), while increasing injection volume (100 µL versus 500 µL; formulation B versus C) led to a prolonged release period. When changing the endcapping from acetyl to propyl, we observed a prolonged release *in vitro* as well as *in vivo*. The same is observed when changing the celecoxib loading from 10 mg/mL (D) to 50 mg/mL (C); the *in vivo* trend of prolonged release was also seen *in vitro*.

For all tested formulations, the *in vivo* release was 2 times faster compared to the *in vitro* situation. As reported previously, this might be due to the presence of enzymes or macrophages at the site of injection resulting in a faster release *in vivo* compared to *in vitro* [25,29–31]. Although we observed similar trends *in vitro* as *in vivo* with changing the different properties of the gels, no level A correlation was obtained (a point-to-point relationship between *in vitro* dissolution and the *in vivo* input rate, which is usually linear [32]). In a previous study, we found that *in vitro*, erosion of the gel (acetyl-endcapped) led to the release of celecoxib and the *in vivo* release was faster due to both the gel depot geometry (larger surface area) as well as the *in vivo* presence of macrophages, leading to a faster degradation of the gel and therefore a faster release of the encapsulated celecoxib [15,26].

3.7. In vivo imaging of tissue distribution of the subcutaneous injected gel

The distribution of the gel after subcutaneous injection was visualized by injecting a near-infrared dye (IR-780 iodide) loaded formulation (20% acetyl endcapped polymer). The physical properties of IR-780 iodide are different compared to celecoxib, but it is a lipophilic compound like celecoxib to mimic the encapsulation of lipophilic compounds in the hydrogel. Fifteen weeks after injection, the animal was sacrificed and the organs were harvested and scanned using fluorescence imaging (Fig. 7).

The scan of the injected subcutaneous area showed that there was still some formulation present 15 weeks post-injection. No accumulation of the formulation was observed in the harvested organs. The only detected signal was coming from the kidneys, which indicates that renal clearance of the formulation as expected is the primary route of excretion as opposed to biliary excretion [33].

4. Conclusion

PCLA-PEG-PCLA thermogels loaded with celecoxib show sustained *in vivo* release up to 8 weeks and are therefore an excellent candidate for sustained local drug delivery. The properties of this thermogel-celecoxib formulation can be altered, leading to tunable release profiles. The apparent *in vivo* half-life of celecoxib was extended from 5 h (bolus injection) to more than 10 days. It was shown that the drug release *in vivo* was about two times faster than *in vitro*, which might be due to faster *in vivo* gel degradation due to the presence of enzymes and or macrophages. Tweaking the gel design (endcapping of the polymer, celecoxib loading or injected volume) leads to different release patterns, with even up to over 8 weeks release period. Therefore, it is possible to steer and even personalize the release based on disease- or patient-specific needs.

Conflict of interest

The authors Paul van Midwoud (employee) and Leo de Leede (consultant) were financially supported by InGell Labs. InGell Labs may financially benefit from this publication, if InGell Labs is successful in marketing products related to this research. Alan Chan is a co-founder of Percuros B.V. and declares that his association with this current manuscript is purely academic and of research interest only.

All other authors declare no conflict of interest.

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