In Vitro and *in Vivo* Characteristics of Celecoxib *in Situ* Formed Suspensions for Intra-Articular Administration

SUSAN WENG LARSEN,[1] ANNA BUUS FROST,[1] JESPER ØSTERGAARD,[1] MAJ HALLING THOMSEN,[2] STINE JACOBSEN,[2] CHRISTIAN SKONBERG,[1] STEEN HONORÉ HANSEN,[1] HENRIK ELVANG JENSEN,[3] CLAUS LARSEN[1]

[1]Department of Pharmaceutics and Analytical Chemistry, Faculty of Pharmaceutical Sciences, University of Copenhagen, DK-2100 Copenhagen Ø, Denmark

[2]Department of Large Animal Sciences, Faculty of Life Sciences, University of Copenhagen, DK-2630 Taastrup, Denmark

[3]Department of Veterinary Disease Biology, Faculty of Life Sciences, University of Copenhagen, DK-1870 Frederiksberg C, Denmark

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ABSTRACT: The objective of the present study was to explore the potential of using an *in situ* suspension forming drug delivery system of celecoxib to provide sustained drug exposure in the joint cavity following intra-articular administration. *In vitro*, precipitates were formed upon addition of a 400 mg/mL solution of celecoxib in polyethylene glycol 400 (PEG 400) to phosphate buffer, pH 7.4, or synovial fluid. The *in vitro* release profiles of the *in situ* formed suspensions were characterized by an initial fast release followed by a slower constant flux. In buffer solutions, these fluxes were comparable to those determined for a preformed suspension containing celecoxib in its most stable crystal form despite the *in situ* formed precipitates contained a mixture of two crystal forms of celecoxib as determined by X-ray powder diffraction. *In situ* suspension formation in synovial fluid was subject to considerable variation. A relatively high dose of celecoxib, corresponding to 1.25 mg/kg, in the form of PEG 400 solution (400 mg/mL) was injected into the radiocarpal joint in four horses. Celecoxib was present in serum samples taken over 10 days and in the joint tissue (post mortem), strongly indicating that joint sustained celecoxib exposure can be achieved using *in situ* suspension formation. © 2011 Wiley-Liss, Inc. and the American Pharmacists Association J Pharm Sci 100:4330–4337, 2011

Keywords: controlled release; formulation vehicle; injectables; *in vitro* models; precipitation; solid state; suspensions

INTRODUCTION

After oral dosing, only a minute fraction of the given drug dose will reach a particular disease-affected joint.[1] For drug substances exhibiting small apparent volumes of distribution (V_D), relative joint exposures of about 0.05% have been reported after intravenous and intra-articular (IA) administration.[2,3] In comparison to oral dosing, local therapeutic concentrations are accomplished at significantly reduced drug doses by injection directly into the joint space, resulting in minimum drug exposure to inappropriate sites. Hence, local administration might be feasible for anti-arthritic drug candidates that are prohibited from oral administration due to severe systemic side effects. Direct IA instillation appears particularly attractive for anti-osteoarthritic drug candidates based on the fact that in osteoarthritis only one or a few joints are affected. Because dissolved small-molecule drugs disappear rapidly from the synovial space, maintenance of therapeutic drug concentrations in the joint over extended periods of time has to be achieved by IA injection of a depot formulation, from which the drug is released in a controlled manner. Joint injection of long-acting (weeks) glucocorticoid suspensions has been common practice for decades.[1] Roughly, duration of action is inversely proportional to the rate of dissolution of the solid particles in the synovial fluid. Depot suspensions may be an advantageous dosage form from the perspective that a high drug load can be achieved and only a minimum of pharmaceutical excipients is needed. However, in spite of the fact that this formulation type is less complex as compared with more advanced

Correspondence to: Claus Larsen (Telephone: +45-35-336466; Fax: +45-35-336030; E-mail: csl@farma.ku.dk)

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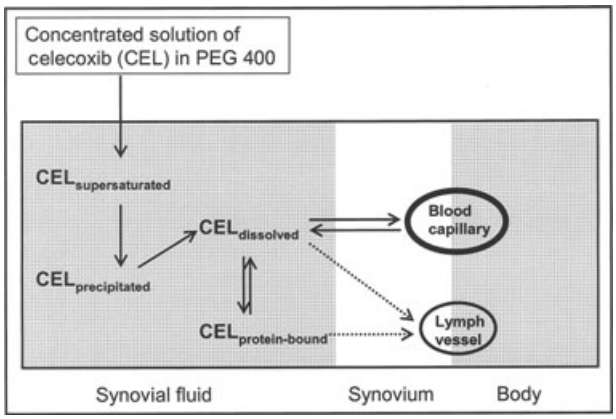

Figure 1. Principle of the *in situ* suspension forming drug delivery system. Intra-articular (IA) injection of a concentrated drug solution, which upon contact with the aqueous synovial fluid (SF), generates the suspension at the site of administration. The sustained drug release properties is influenced by the dissolution rate of the precipitated drug particles, the drug solubility in the synovial fluid, the diffusion through the synovium into the blood capillary, and to a smaller extent transfer into the body via the lymphatic system.

drug delivery systems, parenteral suspensions pose certain challenges with respect to the manufacturing process and the physical stability of the formulation. Hence, *in situ* suspension forming drug delivery systems might constitute a feasible alternative. The latter principle, depicted in Figure 1, involves IA injection of a liquid (preformulation) phase containing the dissolved drug substance, which, upon contact with the aqueous synovial fluid, generates the suspension at the site of administration. The design of *in situ* forming drug delivery systems for parenteral administration has attracted considerable interest.[4] Currently, at least one product (Eligard™ based on the Atrix technology) has been approved by regulatory agencies.[5]

Several poorly water-soluble drug substances might be dissolved in water-miscible cosolvents such as polyethylene glycol 400 (PEG 400). Simple *in situ* suspension formation might be accomplished by adding such organic solutions to an aqueous medium. In the present investigation, this formulation principle was employed to generate and characterize *in situ* formed suspensions of the anti-arthritic drug, celecoxib. Local administration at the site of action of this cox-2 inhibitor is highly relevant because the clinical use of oral celecoxib is currently restricted due to severe side effects.[6] *In vitro* characterization of the formed suspensions included the occurrence of possible polymorphic changes in the drug precipitate as well as determination of drug release characteristics employing the rotating dialysis cell model. Furthermore, based on the analysis of celecoxib in serum and synovial tissue samples, the accomplishment of local sustained celecoxib exposure resulting from joint injection of such PEG 400 solutions of the drug in the horse is reported.

MATERIALS AND METHODS

Materials

Celecoxib was purchased from Cipla (Mumbai, India). PEG 400 was obtained from Sigma–Aldrich (St. Louis, Missouri). Heparin "Leo" 5000 IU/mL was obtained from Leo Pharma (Ballerup, Denmark). Sodium Chloride "SAD" (9 mg/mL), Bupivacaine "SAD" (5 mg/mL), and Lidocaine "SAD" (20 mg/mL) were purchased from Amgros I/S (Copenhagen, Denmark). Deionized water was used throughout the study. Visking dialysis tubing of size 27/32″, 21.5 mm, with a cutoff of 12–14 kDa (VWR International, West Chester, Pennsylvania) was employed for the dialysis cell. Human synovial fluid (SF) from arthritic patients was obtained from the Parker Institute, Frederiksberg Hospital (Frederiksberg, Denmark). Healthy horse SF (collected post mortem) was obtained from the Department of Large Animal Sciences, Faculty of Life Sciences, University of Copenhagen (Copenhagen, Denmark). Other reagents, buffer substances, and solvents were of analytical or reagent grade. A 67 mM phosphate buffer solution (PBS) of pH 7.4 was used in the *in vitro* studies. *In vitro* experiments were also carried out in human and horse SF diluted with PBS to obtain 4:1 (v/v) mixtures (80% SF). All media were adjusted to pH 7.4.

Preparation of the Investigated Suspensions

For *in vitro* characterization, suspensions containing 2.5 mg/mL celecoxib in pH 7.4 PBS or 80% SF in PBS (80% SF) were used unless otherwise stated. The preformed suspensions of celecoxib was prepared by adding solid celecoxib particles directly to the aqueous solutions, whereas the *in situ* suspensions were formed by adding 50 μL of a PEG 400 solution of celecoxib (400 mg/mL) to PBS or 80% SF.

Aqueous Solubility

The solubility of celecoxib in PBS at 37°C was determined ($n = 6$) by adding excess of the drug substance to the medium. The suspensions were rotated at 37 ± 0.5°C in a TH30 incubator hood (Hechingen, Germany) until equilibrium was attained after 3 days, as evidenced by high-performance liquid chromatography (HPLC) analysis of the supernatants sampled at various time points. Also, aliquots of the remaining solid phase (slurries) were investigated by X-ray powder diffraction (XRPD). Furthermore, samples of the solid phase (dried for approximately 5 min at 37°C)

were analyzed by differential scanning calorimetry (DSC).

Solid State Characterization

In Situ Precipitation

The drug precipitation process, initiated by addition of a concentrated celecoxib solution (400 mg/mL) in PEG 400 to PBS or 80% human SF, was followed by XRPD. The *in situ* formed suspensions ($n = 3$) were rotated at $37 \pm 0.5^{\circ}$C in the TH30 incubator hood and samples of the precipitate were taken for XRPD measurements at different time points for the first 24 h.

Determination of Particle Size

Particle size distributions were determined using a laser diffractometer Mastersizer 2000 equipped with a sample dispersion unit (Malvern Instruments, Worcestershire, UK). The volume-based particle size distribution of the investigated celecoxib suspensions was measured ($n = 3$) at room temperature after addition of the suspensions (20 mg/mL celecoxib in PBS) to 150 mL PBS in the sample unit. To ensure reliable measurements, the mean particle diameter of the suspensions was recorded 25 min after the addition of the suspensions. The preformed suspension of celecoxib in PBS was prepared 1 day prior to the particle size determination to ensure that the equilibrium solubility was reached, whereas the *in situ* formed suspension of celecoxib was prepared 2–3 h prior to analysis by adding 50 μL of a PEG 400 solution of celecoxib (400 mg/mL) to 10 mL PBS.

X-ray Powder Diffraction (XRPD)

X-ray powder diffractograms were measured on a PANalytical X'Pert PRO X-Ray Diffractometer (Almelo, The Netherlands) with a PIXcel detector. The Cu Kα1 radiation ($\lambda = 1.5406$ Å) was used for measurements. The voltage and current were 45 kV and 40 mA, respectively. Samples were measured in reflection mode in the 2θ range 2°–40°, with a step size of 0.026° 2θ and a counting time of 213.69 s/step. The automatic divergence slit was used to get a 10 mm irradiated length. Samples were placed on zero background Si disk for analysis, and sample spinning was employed to avoid preferred orientation effect. Data were collected using X'Pert Data Collector software (PANalytical B.V.).

Differential Scanning Calorimetry (DSC)

Differential scanning calorimetry experiments using open pans were performed using Perkin Elmer DSC 7 (PerkinElmer, Norwalk, Connecticut, USA) controlled by Pyris software (version 7.0). Samples of approximately 5 mg celecoxib precipitate were analyzed in 40-μL open pans from 90°C–170°C at a rate of 1°C/min under a nitrogen flow of 20 mL/min.

In Vitro Release Experiments

The release studies were performed at $37 \pm 0.5^{\circ}$C using the rotating dialysis cell model.[7,8] In this model, the donor compartment is separated from the acceptor compartment by a dialysis membrane (interfacial area: 22 cm^2). Experiments were conducted by applying the formulations to the donor compartment containing the following matrices: (i) 67 mM pH 7.4 PBS, (ii) 80% human SF, or (iii) 80% horse SF. At time zero, the dialysis cell containing the suspensions was placed inside a round-bottomed vessel containing 1000 mL of preheated release medium (PBS). The revolution speed of the dialysis cell was 50 rpm. At appropriate times, samples were withdrawn from the acceptor phase and analyzed by HPLC. The preformed suspensions were made by transferring approximately 20 mg celecoxib directly into the donor cell containing 8.0 mL of PBS or 80% human SF + 50 μL PEG 400. *In situ* suspension formation resulted from instillation of 50 μL of a concentrated solution (400 mg/mL) of celecoxib in PEG 400 into 8.0 mL PBS or 80% SF (human or horse). All release experiments were followed for 8–9 h. The cumulated amount of celecoxib released ($M_{A,t}$) was calculated from:

$$M_{A,t} = V_S \sum_{i=1}^{n} C_{i-1} + V_A C_n \quad (1)$$

where V_A and V_S are the volumes of the acceptor phase and the samples withdrawn from the acceptor phase ($V_S = 1.5$ mL), respectively. C_i is the drug concentration in sample i. The samples were diluted with 20% (v/v) acetonitrile to avoid adsorption of celecoxib to the vials used.

In Vivo Experiments

Four adult horses were used in the study (crossbreed). The animals were healthy and free from evidence of joint disease before entering the study. Throughout the study, IA inflammation was assessed daily at least once by clinical evaluation (heat, pain, and swelling) and lameness evaluation. The experimental protocol was preapproved by the Danish Animal Experimentation Board, and all procedures were carried out according to the Danish Animal Testing Act. Sterilization of the investigated celecoxib solution was performed by heat treatment (3 h at 140°C) of the solid celecoxib particles and the PEG 400 solvent prior to aseptic preparation of the final solution of 400 mg/mL celecoxib in PEG 400. A volume of this solution, corresponding to a dose of 1.25 mg celecoxib/kg, was administered intra-articularly by slow injection. Prior to the IA administration, infiltration analgesia of the injection site area was afforded by injection of local analgetics (lidocaine and bupivacaine). Serum samples

were collected from a jugular vein catheter prior to the celecoxib administration, 12 times during the 10-day study period. The samples were stored at –20°C until analysis. Prior to liquid chromatography–tandem mass spectrometry (LC –MS/MS) analysis, the serum samples were deprotenized by addition of 3 volumes of methanol. After centrifugation (14,000 × g , 15 min, room temperature), the supernatant was diluted with acetonitrile–water (1:1 v/v).

Following euthanasia, both radiocarpal joints of all four horses were opened and examined for gross lesions. Specimens of the synovial membrane were sampled for histological evaluation. The tissue specimens were fixed in 10% (v/v) neutral buffered formalin, processed through graded alcohols and xylene, and embedded in paraffin. Haematoxylin and eosin staining was performed on 4–5 µm thick tissue sections.

In addition, the synovial tissue was analyzed for celecoxib residues. Tissue samples were cut into pieces and methanol was added. The mixtures were shaken and left for 20 min. After centrifugation (14,000 × g, 10 min, room temperature), the supernatant was withdrawn and evaporated to dryness over nitrogen. After redissolution in acetonitrile–water (1:1 v/v), the samples were diluted 500 times with ethanol–water (1:1 v/v) prior to LC–MS/MS analysis.

HPLC and LC–MS/MS Analysis

HPLC was performed on a system consisting of a Merck–Hitachi L-6200 pump connected to a Merck–Hitachi L-4000 UV detector, a Merck–Hitachi L-7200 autosampler, and a Peltier Sample Cooler (30°C) (VWR International, Tokyo, Japan). Reversed phase chromatography was performed using a C18 Gemini® RP column (150 × 4.6 mm, 5 µm particles) (Phenomenex, Torrance, California) equipped with a SecurityGuard precolumn (Phenomenex). The flow rate was set to 1 mL/min and the column effluent was monitored at 254 nm. The mobile phase consisted of 60% (v/v) acetonitrile and 40% (v/v) of 0.1% (v/v) phosphoric acid. Quantification of celecoxib was performed from peak area measurements in relation with those of standards chromatographed under the same conditions.

Liquid chromatography–tandem mass spectrometry was performed using a Thermo Finnigan Surveyor HPLC system (Thermo Finnigan) coupled to a Thermo Finnigan TSQ AM Ultra triple quadrupole mass spectrometer with an ESI IonMax interface (Thermo Finnigan) and a Thermo Hypersil GOLD column (50 × 2.1 mm, 1.9 µm particles) (Thermo Electron Corporation, Copenhagen, Denmark) with the column oven set at 40°C. MS-system settings: negative electrospray-ionization settings: voltage 4000 V; sheath gas 55 (arbitrary unit (arb)); auxiliary gas 20 (arb); sweep gas 4.0 (arb); capillary temperature 350°C; skimmer offset 16 V. Single ion monitoring settings: isolation *m/z* 380.10; isolation width 0.50 unit; scan time 0.05 s; Q1 peak width 0.5 FWHM (full with half at half maximum). MS/MS settings: isolation on *m/z* 380.10; collision energy 32; Q2 gas pressure 1.5; scan *m/z* 30–425; scan time 0.25 s, Q1 and Q3 peak width set to 0.5 FWHM. Gradient elution was employed using mobile phase A: Milli-Q water with 0.05% (v/v) formic acid, and mobile phase B: acetonitrile with 0.05% (v/v) formic acid. The gradient was as follows: 0–4 min, 40%–100% B; 4–5 min, 100% B; 5–5.5 min, 100%–40% B; and 5.5–9 min, 40% B. The flow rate was 250 µL/min. The limit of quantification (LOQ) was found to be approximately 5 ng/mL. Relative standard deviations of 10%–15% at LOQ were accepted due to carry over problems.

RESULTS AND DISCUSSION

In the present study, *in vivo* and *in vitro* performances of an *in situ* formed suspension of celecoxib intended for IA administration were investigated. The present sustained release principle involves (i) celecoxib precipitation in the aqueous synovial fluid after IA injection of a concentrated celecoxib solution in PEG 400 and (ii) prolonged local drug exposure resulting from slow dissolution of celecoxib in the synovial fluid (Fig. 1). PEG 400 was selected as cosolvent for these experiments due to the high solubility of celecoxib (>400 mg/mL) in this solvent.[9] Furthermore, PEG 400 is considered to be a relatively safe cosolvent.[10]

In Vitro Studies Aqueous Solubility

Four polymorphic forms of celecoxib have been reported where crystal form III (The Cambridge Structural Database reference code: DIBBUL; single crystal structure reported by Dev et al.[11]) is the thermodynamically stable form of the drug substance at ambient temperature.[12] XRPD and DSC analyses confirmed that the celecoxib batch used in the present study contained form III. The solubility of celecoxib in PBS was found to be 1.7 µg/mL at 37°C [relative standard deviation (RSD) = 4.3%], which is in agreement with the previously reported solubilities.[9,13] A drug concentration almost three times that of the saturation solubility (determined at equilibrium) was determined up to about 2 h upon addition of excess celecoxib to PBS. At different time points (from 10 min to 24 h), celecoxib slurries were characterized by XRPD and DSC measurements. In all cases, the precipitate was found to consist of crystal form III of celecoxib. Therefore, the observed initial solubility behavior cannot be ascribed to the occurrence of polymorphic transformations during the solubility experiment. Hence, the initial supersaturation observed could be explained by the presence of minute amounts of (i) amorphous celecoxib or (ii) very small crystalline celecoxib particles in the bulk substance.

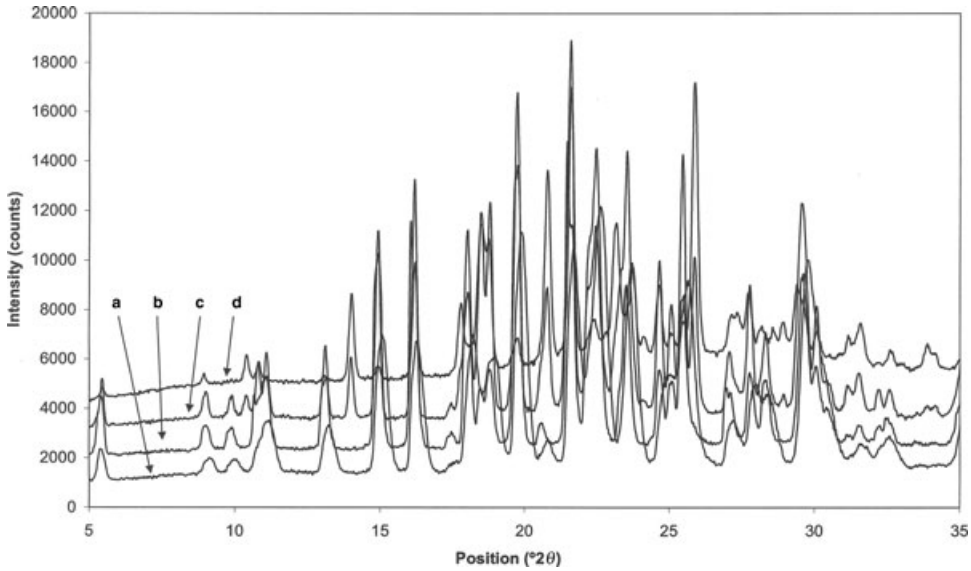

Figure 2. X-ray powder diffractograms of celecoxib crystal form III (a), solid celecoxib particles from preformed suspension in PBS (b), precipitated celecoxib from *in situ* formed suspension in PBS (c), precipitated celecoxib from *in situ* formed suspension in 80% (v/v) human SF (d).

Characterization of the *in Situ* Formed Precipitate

The precipitate formed upon addition of a PEG 400 solution of celecoxib (400 mg/mL) to PBS or 80% human SF was investigated by XRPD. The diffractograms of the precipitated material (sampled at different time points) deviated from those obtained from celecoxib bulk substance (form III) (Fig. 2). In addition to form III, the precipitates formed in the two matrices contained another polymorphic form of celecoxib. The presence of extra reflections corresponding to those previously observed for crystalline form II of celecoxib[12,14] suggests that the precipitates were mixtures of celecoxib form II and III. Determination of the particle size distribution of the *in situ* formed precipitate in PBS revealed a larger peak and a minor peak with mean diameters of about 10 and 250 μm, respectively. In comparison, a particle size distribution of the preformed celecoxib suspension in PBS was characterized by $d(0.1) = 4.7\,\mu m$, $d(0.5) = 12.5\,\mu m$, and $d(0.9) = 45.7\,\mu m$. The fraction of larger particles observed for the *in situ* formed precipitate in PBS is most likely caused by celecoxib particle aggregation. In contrast, large particles were not observed by visual inspection of the precipitate formed by addition of concentrated PEG solution of celecoxib to 80% human SF. Particle sizes in SF could not be measured due to interference of the biological matrices.

In Vitro Release

The rotating dialysis cell model has been proposed to be useful for the study of events influencing drug residence time in the joint cavity after IA instillation of depot injectables.[8,15–17] In the case a suspension is formed in the small aqueous donor compartment, the rate of drug appearance in the acceptor phase is governed by (i) the rate of dissolution of the solid particles in the aqueous donor phase and/or (ii) the rate of diffusion of drug across the membrane into the acceptor phase. In case of small-molecule drugs, the rate of the two processes increases with increasing solubility of the drug compound. In the present study, the sustained release characteristics of the *in situ* formed suspension of celecoxib were compared with those of the preformed celecoxib suspension. In the donor compartment, a high drug load (20 mg celecoxib added to 8.0 mL of aqueous donor medium) was added to ensure sufficient excess of celecoxib. All release data presented are generated under sink condition, which in the present study is defined as drug concentrations below one-tenth of the saturation solubility.

For the preformed and *in situ* formed suspensions in PBS, plots of the cumulated amounts of celecoxib released into the acceptor phase versus time are depicted in Figure 3. The release profiles are characterized by an initial fast release of celecoxib, after which constant fluxes are observed. For the preformed suspension, the time period of the fast drug release coincides with the initial phase of the solubility experiment characterized by the existence of a supersaturated solution of celecoxib. This phenomenon may be explained by the presence of a small amount of amorphous celecoxib in the bulk drug substance, which was not detectable by XRPD. Considering the release profile of the *in situ* formed suspension, the initial phase of fast drug release may be ascribed to formation of a supersaturated solution as a result of incomplete precipitation of celecoxib. Similar release behavior has

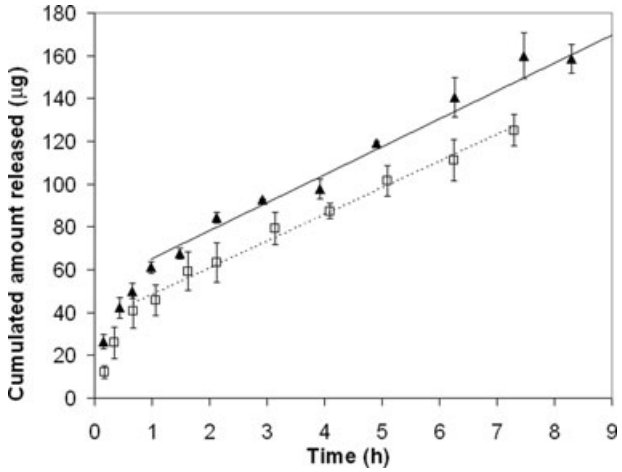

Figure 3. Release profiles obtained in the rotating dialysis cell model at 37°C after applying a preformed celecoxib suspension (□) and a concentrated solution of celecoxib in PEG 400 (*in situ* formed suspension) (▲) to the donor cell containing PBS (pH 7.4). Experiments were performed in triplicate and error bars represent SD. The straight lines refer to the constant fluxes.

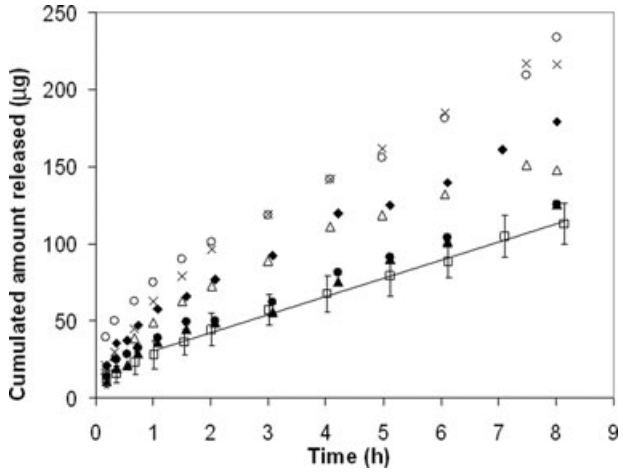

Figure 4. Release profiles obtained in the rotating dialysis cell model at 37°C after applying a preformed celecoxib suspension (□; $n = 3$) and a concentrated solution of celecoxib in PEG 400 (*in situ* formed suspension) (▲,●,○,×,◆,Δ,+,Ú; single experiments) to the donor cell containing 80% human SF (pH 7.4). Error bars represent SD. The straight line refers to the constant flux.

been reported in studies of *in situ* formed suspensions of lidocaine and bupivacaine.[18,19] A prerequisite for the observed steady state fluxes is the maintenance of a constant celecoxib concentration in the aqueous donor compartment.[16,20] Identical constant fluxes were found for the preformed (0.57 µg cm^{-2} h^{-1}, $n = 3$, RSD = 7.2%) and *in situ* formed (0.59 µg cm^{-2} h^{-1}, $n = 3$, RSD = 3.5%) suspension systems. These observations indicate that equal steady state concentrations of celecoxib have been attained in the two suspension systems. Thus, under the present experimental conditions, differences in the particle size distribution do not affect the release profiles. From the release data, it is not known whether the *in situ* formed precipitate contains a mixture of crystal form II and III as was found for the *in situ* celecoxib precipitate formed in a separate experiment (see the above subsection) because XRPD measurements of the solid material in the donor cell as a function of time were not possible.

In Figure 4, plots of the cumulated amounts of celecoxib released into the acceptor phase versus time for the preformed and *in situ* formed suspensions in 80% human SF are presented. In SF, the preformed suspension showed drug release characteristics comparable to those determined in PBS (flux = 0.53 µg cm^{-2} h^{-1}, $n = 3$, RSD = 7.5%). In contrast, the release data for the *in situ* formed suspension in the human SF were subject to considerable variation. The duration of the initial phase of fast release varied from 1 to 2 h and the obtained fluxes were subject to significant fluctuations (flux = 0.70 µg cm^{-2} h^{-1}, $n = 8$, RSD = 24%). Interestingly, replacement of 80% human SF by 80% horse SF resulted in significant faster drug disappearance from the donor cell (flux = 3.59 µg cm^{-2} h^{-1}, $n = 3$, RSD = 24%). This observed alteration in release characteristics might relate to qualitative and quantitative differences in composition as well as content of proteins in the synovial fluids taken from healthy horses and arthritic patients, respectively. Attempts to study the differences between the two biological matrices were not made due to limited access to SF from healthy horses. The variability in the release behavior observed in the biological matrices might indicate that the precipitation process (rate and extent) is kinetically controlled. Inhibition of drug precipitation by macromolecules such as polymers from supersaturated solutions has previously been observed for several drug substances,[21] including celecoxib.[22] Also, the ability of celecoxib to interact strongly with proteins might affect the precipitation process.[23] Though the performed *in vitro* experiments indicate the utility of the concept of *in situ* suspension formation, factors influencing *in situ* precipitation of celecoxib in biological matrices have not been fully elucidated.

In Vivo Experiments Celecoxib Detection in Serum and Joint Tissue

To each of four horses, a dose of celecoxib corresponding to 1.25 mg/kg was injected into the radiocarpal joint in the form of concentrated PEG 400 solution (400 mg/mL). The injected volumes were in the range of 1.1–1.7 mL. Because of the shortage of knowledge on the IA half-life of celecoxib, a relatively high dose

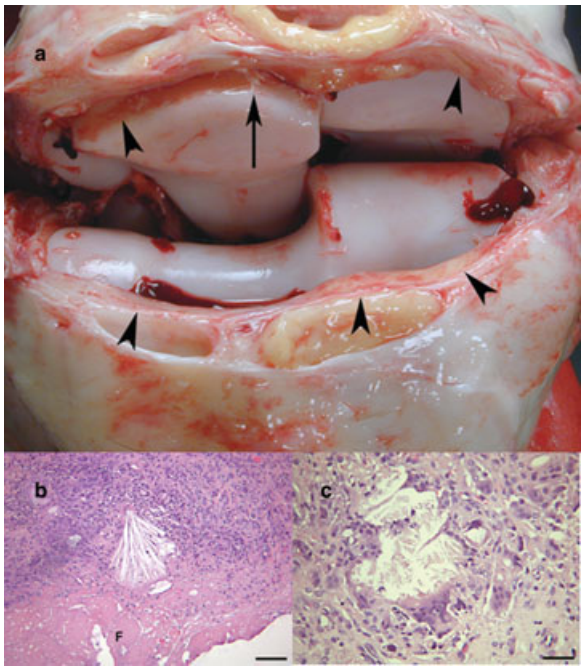

Figure 5. Equine radiocarpal joint 10 days after IA injection of a concentrated PEG 400 solution of celecoxib corresponding to a dose of 1.25 mg/kg. (a) The synovial membrane is diffusely thickened and hyperemic (arrow heads). Focally between the articular cartilage and synovial membrane white-gray, foreign material (arrow) is seen. (b) Synovial membrane (100× magnification). Toward the synovial cavity, a rim of fibrin with a few neutrophils is seen (denoted by F). Between the layer of fibrin and the synovial membrane, an area with crystalline material is situated. Throughout the synovial membrane, a granulomatous inflammation is present and is accompanied by proliferation of capillaries. Bar = 75 μm. (c) Synovial membrane (250× magnification). Multinucleated giant cells are formed around the crystalline material. Bar = 30 μm.

was selected to enable detection of the drug in the systemic circulation over an extended period of time. Analysis of the serum samples revealed detectable (>1 ng/mL) celecoxib concentrations in serum from all samples taken over a time period of 10 days. Most of the samples contained celecoxib levels above LOQ (5 ng/mL). The presence of small concentrations of celecoxib in the general circulation over the entire study period is in accordance with the observation that post-mortem joint tissue samples contained solid residues of celecoxib. Thus, the data strongly indicate that the *in situ* formed suspension has afforded joint tissue exposure of low celecoxib concentrations over a 10-day period. The low and variable celecoxib plasma concentrations were prohibitive for a strict pharmacokinetic analysis.

Preliminary Toxicological Observations

The four horses did not show any clinical signs of lameness or joint inflammation in response to IA injection of celecoxib. In all injected joints, the synovial membrane was enlarged and hyperaemic and small masses of white-gray foreign material were situated in the area between the articular cartilage and the synovial membrane (Fig. 5a). Histologically, the synovial membranes of all the injected joints were in some areas covered with a thin rim of fibrin. In other surface areas, a crystalline material was present too (Fig. 5b). The synovial lining cells were diffusely hypertrophic and hyperplastic. Mononuclear cells, especially macrophages, heavily infiltrated the synovial membrane, but also lymphocytes and a few plasma cells were present. Throughout the synovial membrane, multiple giant cells had formed; and within the giant cells as well as surrounded by the macrophages, a crystalline material similar to that observed on the surface of the synovial membrane, was present (Fig. 5c). The synovial membrane of all control joints appeared histologically normal (data not shown). The observed white-gray foreign material in the injected joints was identified as celecoxib by LC–MS/MS analysis, and the gross and histological observations were comparable to a granulomatous foreign body reaction toward the drug. Whether similar host reactions will be elicited with smaller and perhaps more clinically relevant drug doses warrants further investigation.

CONCLUSION

The present study has shown that an *in situ* suspension forming drug delivery system containing celecoxib can provide local sustained drug exposure following IA administration. The precipitate formed upon addition of a concentrated solution of celecoxib in PEG 400 to a buffer solution (pH 7.4) contained a mixture of two polymorphic forms of celecoxib; however, the *in vitro* release behavior of the *in situ* formed suspension was comparable to that obtained for the preformed suspension containing only one crystal form. *In situ* suspension formation of celecoxib *in vitro* was also observed in human and horse SF. Under these conditions, the precipitation process was subject to considerable variation. Local sustained release following IA injection of the PEG 400 solution of celecoxib in the horse was demonstrated by the presence of celecoxib in the systemic circulation over a 10-day period and the detection of celecoxib in the joint tissue post mortem (after 10 days). Preliminary toxicological observations revealed the presence of granulomatous synovitis 10 days after IA injection of a relatively high celecoxib dose (1.25 mg/kg) in the form of concentrated PEG 400 solution (400 mg/mL). Further studies of *in situ* suspension forming drug delivery systems are ongoing in our laboratory, with focus on factors influencing the precipitation process.

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