Journal of Controlled Release 209 (2015) 20–26

Contents lists available at ScienceDirect

Journal of Controlled Release

journal homepage: www.elsevier.com/locate/jconrel

[18]F FDG PET/CT imaging to monitor the therapeutic effect of liposome-encapsulated prednisolone in experimental rheumatoid arthritis

Tessa van der Geest [a,*], Josbert M. Metselaar [b,c], Danny Gerrits [a], Peter L. van Lent [d], Gert Storm [e], Peter Laverman [a], Otto C. Boerman [a]

[a] Department of Radiology and Nuclear Medicine, Radboud University Medical Center, Geert Grooteplein zuid 10, 6525 GA Nijmegen, The Netherlands
[b] Department of Targeted Therapeutics, MIRA Institute, University of Twente, Zuidhorst, 7500 AE Enschede, The Netherlands
[c] Department of Experimental Molecular Imaging, University Clinic and Helmholtz Institute for Biomedical Engineering, RWTH-Aachen University, Pauwelstrasse 30, 52074 Aachen, Germany
[d] Department of Experimental Rheumatology, Radboud university medical center, Geert Grooteplein 28, 6525 GA Nijmegen, The Netherlands
[e] Department of Pharmaceutics, Utrecht Institute for Pharmaceutical Sciences, Utrecht University, Utrecht, The Netherlands

ARTICLE INFO

Article history:
Received 16 March 2015
Received in revised form 14 April 2015
Accepted 15 April 2015
Available online 19 April 2015

Chemical compounds studied in this article:
Cholesterol (PubChem CID: 5997)
Dipalmitoylphosphatidylcholine (PubChem CID: 452110)
1,2-Distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000] (CID 406952)
Fluorodeoxy glucose (PubChem CID: 450503)
Prednisolone Phosphate (PubChem CID:5736945)

Keywords:
Antigen-induced arthritis
[18]F]FDG
PET/CT
Liposomes
Glucocoticoids
Therapy monitoring

ABSTRACT

Current treatment of rheumatoid arthritis includes systemic administration of glucocorticoids. To improve joint targeting and anti-inflammatory efficacy these glucocorticoids are encapsulated in long-circulating liposomes. The present study aimed to monitor therapeutic effects of prednisolone (PLP)-containing PEG-liposomes in murine antigen-induced arthritis (AIA) using [18F]FDG PET/CT.

Mono-articular arthritis was induced in male C57Bl6/J mice. At 0, 3, 7 and 12 days after arthritis induction, inflamed joints were macroscopically scored (0 = unaffected to 4 = immobile) and [18F]FDG PET/CT images were acquired. In a second experiment, to study the feasibility to monitor therapeutic effects of PLP encapsulating PEG-liposomes, mice were treated with a single i.v. injection of PLP-containing PEG-liposomes (10 mg/kg) or empty PEG-liposomes 3 days after arthritis induction. Inflamed joints were macroscopically scored and images were acquired at −3, 0, 4 and 9 days after treatment. PET images were analyzed quantitatively, and mice were dissected to allow histological analysis of the joints. With progression of arthritis, [18F]FDG uptake in inflamed joints increased significantly (day 0: 2.5 ± 0.9% ID/ml, day 7: 4.4 ± 0.4% ID/ml, $p = 0.0159$), while no changes were observed in unaffected paws (day 0: 2.5 ± 1.1% ID/ml, day 7: 2.7 ± 0.8% ID/ml, $p = 0.3466$). In the second experiment, macroscopic scoring revealed suppression of joint swelling after treatment with PLP-containing PEG-liposomes. In line with that, [18F]FDG uptake did not change in the treated mice (day −3: 1.9 ± 0.3% ID/ml, day 4: 2.2 ± 0.2% ID/ml, $p = 0.3466$), while it increased in mice that developed arthritis (day −3: 2.0 ± 0.2% ID/ml, day 4: 3.1 ± 0.6% ID/ml, $p = 0.0225$). Histological analysis confirmed therapeutic efficacy, which showed less inflammation ($p = 0.0354$) and bone erosion ($p = 0.0298$) in treated mice. These data show that [18F]FDG PET/CT could be used to monitor the progression of AIA and confirmed rapid and profound anti-inflammatory effects of PLP-containing PEG-liposomes that were also observed macroscopically and microscopically.

1. Introduction

Rheumatoid arthritis (RA) is one of the most common autoimmune disorders, affecting approximately 1% of the adults in the western world [1]. It is a chronic, systemic and progressive disease, characterized by joint inflammation and cartilage destruction [2]. Though effective medication has gradually become available since the middle of the previous century, RA is still incurable [3]. Glucocorticoids (GC) were among the first and most important and still retain a prominent place in the therapy of RA [4]. However, the issue of poor accumulation at target sites and awareness of potential side effects has limited the application in the field. High and frequent dosing is often required to treat severe chronic inflammation, which might lead to well-known severe adverse effects as Cushing syndrome, induction of diabetes, osteoporosis, muscular atrophy and thinning of the skin [5,6].

To enhance local therapeutic activity and reduce systemic side effects, various approaches have been developed to improve GC targeting

* Corresponding author at: Radboud University Medical Center, Department of Radiology and Nuclear Medicine, P.O. Box 9101, 6500 HB Nijmegen, The Netherlands.
E-mail addresses: Tessa.vanderGeest@radboudumc.nl (T. van der Geest), j.m.metselaar@utwente.nl (J.M. Metselaar), Danny.Gerrits@radboudumc.nl (D. Gerrits), Peter.vanLent@radboudumc.nl (P.L. van Lent), g.storm@uu.nl (G. Storm), Peter.Laverman@radboudumc.nl (P. Laverman), Otto.Boerman@radboudumc.nl (O.C. Boerman).

http://dx.doi.org/10.1016/j.jconrel.2015.04.019
0168-3659/

to inflamed joints. A local strategy is intra-articular therapy, where GC are directly injected in the inflamed joint. This approach is effective, but not applicable when targeting multiple afflicted joints, as is the case in polyarthritis, where systemic delivery would be preferable. A systemic approach is delivery of GC by encapsulation in liposomes. These lipid vesicles can encapsulate hydrophilic agents in their aqueous core. Encapsulation of GC in liposomes has proven to result in improved pharmacokinetics, better targeting to inflamed sites, reduced metabolism of the GC and less side effects.

Long-circulating liposomes (LCL) are small (ca. 100 nm diameter) vesicles that consist of a lipid bilayer of phospholipids, cholesterol and are coated with polyethylene glycol (PEG). PEG sterically hinders plasma protein binding which protects them from being opsonized and prematurely phagocytised by macrophages, and therefore exhibit prolonged circulation times and limited hepatic excretion [7]. It has been shown that the leaky vessels in inflamed and cancerous tissue are permeable for colloidal formulations with diameters up to 200 nm [8,9].

In arthritis, the inflamed synovial area contains activated macrophages. These macrophages can process drug-containing LCL, which leads to local drug release. Local drug release prevents non-target effects and widens the therapeutic window of the drug [10]. Prednisolone (PLP) is an immunosuppressive drug used to treat RA. It has been shown in preclinical as well as in clinical studies that treatment of experimental arthritis with PLP-containing LCL, is markedly more effective than pulse therapy or intra-articular injections of free PLP [11–14].

Typically, progression of experimental arthritis and therapeutic effects on the model are assessed by clinical scoring (visual) and histopathological evaluation of the joints. However, with imaging modalities such as positron emission tomography (PET) or single-photon emission computed tomography (SPECT) the severity of inflammation may be assessed in a novel non-invasive way and potentially the therapeutic effect can be monitored [15,16]. It has been demonstrated that the accumulation of [^{18}F] fluorodeoxyglucose (FDG) increases with the progression of joint swelling and reflects the characteristic changes in pathological progression [17,18]. There is early evidence that [^{18}F]FDG positron emission tomography/computed tomography ([^{18}F]FDG PET/CT) imaging can be used assess disease activity in RA. In the present study we provide more evidence that this is also the case for disease progression in mice with antigen-induced arthritis (AIA). Additionally, we investigated whether the therapeutic effect of PLP-encapsulating LCL can be monitored in mice with AIA.

2. Materials and methods

2.1. Preparation of liposomes

Empty LCL and LCL containing 2.1 mg/ml PLP were prepared by injection of an ethanolic lipid solution into an aqueous dispersion medium, followed by extrusion, as described earlier [11]. Briefly, dipalmitoylphosphatidylcholine (DPPC), 1,2-distearoylphosphatidylethanolamine-methyl-polyethyleneglycol conjugate-2000 (mPEG2000-DSPE) (both from Lipoid GmbH, Ludwigshafen, Germany) and cholesterol (BUFA, Uitgeest, The Netherlands) (1.85: 0.15: 1 M ratio) were dissolved in ethanol. The aqueous drug solution contained 100 mg/ml PLP (BUFA, Uitgeest, The Netherlands) in water for injection or, in case of the empty LCL, saline (B. Braun, Melsungen, Germany). The ethanolic lipid solution was injected in the aqueous solution and the resulting coarse dispersion was downsized by multiple extrusion steps through polycarbonate filter membranes with decreasing pore sizes of 200, 100 and 50 nm. Size (about 110 nm) and mean polydispersity (about 0.07) were determined by dynamic light scattering using a Malvern 4700 system (Malvern Ltd, Malvern, UK). The polydispersity index is a measure for the heterogeneity of particles in solution. Unencapsulated PLP was removed by dialysis against saline using Slide-A-Lyzer dialysis cassettes (Thermo Fisher Scientific, Etten-Leur, The Netherlands) with a molecular weight cut-off of 10 kD and repeated changing of the dialysis medium (PBS).

2.2. Animals

Male C57Bl/6J mice (8–12 weeks old) were purchased from Janvier Labs (le Genest-Saint-Isle, France). Mice were housed under standard laboratory conditions (temperature, 20–24 °C; relative humidity, 50–60%; 12 h light–dark cycle) and had food (SNIFF Voer, Soest, The Netherlands) and water available ad libitum. All animals were accustomed to the environment for at least one week before experiments were initiated. All in vivo experiments were approved by the Institutional Animal Welfare Committee of the Radboud university medical center, Nijmegen, and were conducted in accordance with the principles laid out by the revised Dutch Act on Animal Experimentation (1997).

2.3. Antigen-induced arthritis (AIA)

AIA was induced as described previously [19]. Three weeks prior to induction of arthritis, mice were immunized with 100 μg methylated bovine serum albumin (mBSA, 1 mg/ml, Sigma-Aldrich, St. Louis, USA), emulsified in Complete Freund's Adjuvant (CFA, Difco Laboratories, Detroit, USA), which was injected intradermally into the flanks and the footpad of the forelegs. Heat-killed *Bordetella Pertussis* (RIVM, Bilthoven, The Netherlands) was administered intraperitoneally as an additional adjuvant. After 7 days, two intradermal booster injections of 50 μg mBSA/CFA (1 mg/ml) were administered in the neck region of the mice. Finally, arthritis was induced by a single intra-articular injection of 60 μg of mBSA in PBS (10 mg/ml) into the right knee joint. The left knee joint remained untreated and served as an internal control.

2.4. Monitoring progression of arthritis

AIA was induced in four groups of male C57Bl6/J mice (n = 6 per group). At 0, 3, 7 and 12 days after induction of arthritis one group underwent [^{18}F]FDG PET/CT imaging. Mice were anesthetized using 1.5–3% isoflurane in air during injection of the tracer, during the uptake phase of the tracer and during image acquisition. During anesthesia mice were placed on a heating pad to maintain their body temperature, and eyedrops (Opthtosan, AST Farma b.v., Oudewater, The Netherlands) were administered to prevent dehydration of the eyes.

Mice were euthanized after image acquisition by CO_2/O_2 suffocation.

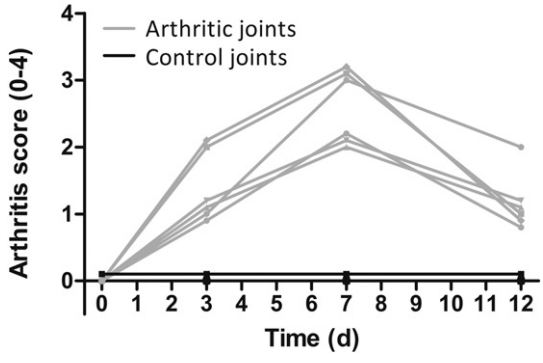

Fig. 1. Arthritis scores in time of arthritic joints (right knee, gray lines) and control joints (left knee, black lines) of C57Bl6/J mice during the development of arthritis. Knee joint swelling was macroscopically scored, scores ranged from 0–4: 0 = Unaffected, 1 = Mild swelling, no difficulties in mobility, 2 = Swollen knee, impaired mobility, 3 = Severe swelling, no use of affected paw, 4 = Severe swelling, immobility. Score in arthritic knee day 0 = 0, day 3 = 0–2, day 7 = 2–3, day 12 = 1–2, in control joints all time points = 0 (Wilcoxon matched-pairs signed rank test, p = 0.002).

2.5. Monitoring therapeutic efficacy of PLP-containing LCL

AIA was induced in two groups of male C57Bl6/J mice (n = 6 per group) as described above. Mice were treated 3 days after arthritis induction. One group of mice received PLP-containing LCL in PBS (10 mg/kg body weight, 200 μl i.v.), while the other group received empty LCL in PBS (200 μl i.v.). Effect of the treatment was monitored using [^{18}F]FDG PET/CT at 3, 7 and 12 days after induction of arthritis. Severity of inflammation was evaluated at each time point. Knee joint swelling was macroscopically scored, scores ranged from 0–4: 0 = joint is unaffected, 1 = joint is mildly swollen, but the mouse shows no difficulties in mobility, 2 = joint is swollen, and the mouse shows impaired mobility, 3 = joint is severely swollen, mouse did not use the affected paw, 4 = joint severely swollen, the mouse becomes immobile.

After image acquisition mice were euthanized and knee joints were isolated and fixed for 4 days in 10% buffered formalin, decalcified in 5% formic acid for 1 week, dehydrated and embedded in paraffin for histological analysis.

2.6. [^{18}F]FDG PET/CT image acquisition

PET/CT imaging was conducted with an Inveon small-animal PET/CT system (Siemens Preclinical Solutions, Knoxville Tennesse, USA). Whole body PET images were obtained 45 min after i.v. administration of 10 ± 2.5 MBq [^{18}F]FDG via the tail vein. PET scans were performed for 15 min followed by a CT scan for anatomic reference (spatial resolution, 113 μm; 80 kVp; and 500 μA). Acquired data were reconstructed iteratively in a 256 × 256 × 159 matrix using an attenuation-weighed 3-dimensional ordered-subsets expected maximization/fast maximum a posterior (OSEM3D/fMAP) algorithm using Inveon Acquisition Workplace software (version 1.5; Siemens Preclinical Solutions, Knoxville Tennessee, USA. Scans were analyzed using Inveon Research Workplace 4.1 Software (Siemens Preclinical Solutions, Knoxville Tennessee, USA). Spherical isocontours were set at 50% of the maximal pixel value to measure uptake values (Bq/ml) in inflamed (right) and unaffected (left) knee joints. Bq/ml$_{mean}$ in inflamed joints and unaffected joints were used to calculate the %ID/ml. To determine the %ID/ml. Bq/ml$_{mean}$ was corrected for the amount of injected activity and time between

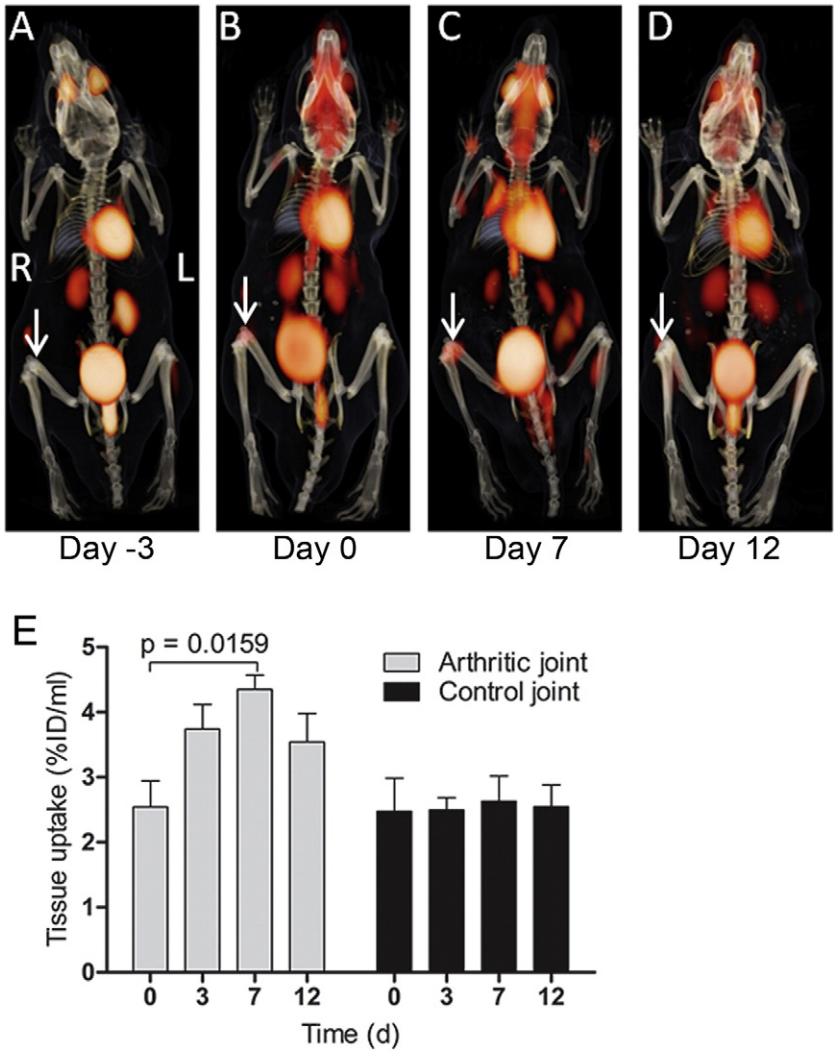

Fig. 2. [^{18}F]FDG PET/CT imaging (45 min p.i.) demonstrated [^{18}F]FDG accumulation in the affected knee joint during the development of AIA. Representative [^{18}F]FDG PET/CT images of a mouse at day 0 (A), day 3 (B), day 7 (C) and day 12 (D) after induction of arthritis in the right knee joint (arrows). Radioactivity was seen in the heart, kidneys, bladder and brain. E: Quantification of the tissue uptake of [^{18}F]FDG (%ID/ml) in arthritic joints and control joints, based on [^{18}F]FDG PET/CT images, during the progression of arthritis. A significant increase in [^{18}F]FDG uptake was found at day 7 after induction of arthritis at day 0 (p = 0.0159). Graph values represent mean ± SD, n = 6 mice per group. The significance of difference was tested by the Mann–Whitney *U* test.

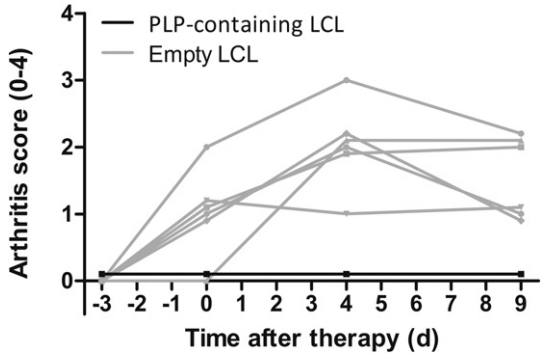

Fig. 3. Arthritis scores in time of arthritic joints (right knee) of mice that received either PLP-containing LCL (black lines) or empty LCL (gray lines) at day 0. Knee joint swelling was macroscopically scored, scores ranged from 0–4: 0 = Unaffected, 1 = Mild swelling, no difficulties in mobility, 2 = Swollen knee, impaired mobility, 3 = Severe swelling, no use of affected paw, 4 = Severe swelling, immobility. Score in arthritic knee of treated mice: all time points: 0 and mice that received empty LCL: day −3: 0, day 0: 0–2, day 4: 1–3, day 9: 1–2 (Wilcoxon matched-pairs signed rank test, p = 0.0310).

injection and image acquisition, in which the radionuclide decayed. Quantification of the PET signal was performed by an independent technician not aware of the treatment groups.

2.7. Histology

Standard frontal knee sections (7 μm) were mounted on Superfrost slides (Menzel-Gläser, Braunschweig, Germany) and stained with hematoxylin and eosin (HE). Synovial inflammatory activity and bone erosion was examined in HE-stained frontal sections of whole knee joints of mice that were dissected at day 7. Severity of inflammation was determined as described previously [20], by scoring the cellular infiltration of into the synovium using an arbitrary scale between 0 and 3, 0 = no cells, 1 = mild cellularity, 2 = moderate cellularity, 3 = maximal cellularity. Three representative sections per knee joint were scored and scoring was performed by two blinded observers.

2.8. Statistical analysis

Statistical analysis was performed using SPSS software version 20 (IBM, Chicago, IL, USA). Mean values are given ± standard deviations. Statistical analysis of [^{18}F]FDG uptake in the joints and histological evaluation were calculated using nonparametric, Mann–Whitney *U* tests. Statistical analysis of arthritis scores was performed using nonparametric, Wilcoxon matched-pairs signed ranked tests. All tests were two-sided and a p-value of 0.05 was considered significant.

3. Results

3.1. Monitoring progression of arthritis

Antigen-induced arthritis (AIA) was induced by an intra-articular injection of mBSA in the right knee joint of pre-immunized mice, the left knee joint served as a negative control. Macroscopic scoring of the joints, which was based on joint swelling and mobility of the mice, showed the 7-day course of AIA. Arthritis scores of arthritic joints increased significantly from day 0 and peaked at day 7, while no changes were observed in unaffected control joints (Fig. 1, score day 0 = 0, day 3 = 0–2, day 7 = 2–3, day 12 = 1–2, Wilcoxon matched-pairs signed rank test p = 0.002).

[^{18}F]FDG PET/CT images were acquired of all mice at day 0, day 3, day 7 and day 12 after the intra-articular induction of arthritis. A representative set of microPET/CT images is shown in Fig. 2A–D. All images were analyzed quantitatively to determine the [^{18}F]FDG accumulation. Activity values in volumes of interest (VOI) drawn around the inflamed joints and control joints at day 0, day 3, day 7 and day 12 after induction of arthritis, were converted to %ID/ml and are presented in Fig. 2E. [^{18}F]FDG PET/CT imaging revealed increased accumulation of [^{18}F]FDG up to day 7 in the right knee (arthritic joint) of the mice, while no increase in [^{18}F]FDG uptake was seen in the left knee (control joint, Fig. 2). Additionally, activity was seen in heart, kidneys, bladder and brain. Quantification of this [^{18}F]FDG accumulation confirmed increased uptake in inflamed joints with progression of arthritis. Maximum uptake was measured at day 7 (Mann Whitney *U*, p = 0.0159), while no changes were observed in control joints (Mann Whitney *U*, p = 0.3466).

3.2. Monitoring therapeutic effect PLP-containing LCL with [^{18}F]FDG PET/CT

Mice with AIA were injected i.v. at day 3 after induction of arthritis, prior to imaging, either with a single injection of 10 mg/kg PLP-containing LCL in PBS or empty LCL in PBS. Joint swelling was macroscopically monitored during the course of arthritis and was completely suppressed in the treated group, while empty LCL did not have a suppressive effect on joint swelling (Fig. 3).

At day −3, 0, 4, and 9 after treatment, [^{18}F]FDG PET/CT images were acquired. [^{18}F]FDG PET/CT imaging showed no uptake in the right knee, in which arthritis was induced, of treated mice, while [^{18}F]FDG uptake increased up to day 4 in arthritic knees of mice that received empty LCL. Fig. 4 shows images acquired at day −3 and day 4 of mice that were treated with PLP-containing LCL (A and B) and mice that received empty LCL (C and D). In addition to the [^{18}F]FDG uptake in the arthritic knees, [^{18}F]FDG accumulated in the front paws. This [^{18}F]FDG uptake also decreased after treatment with PLP-containing LCL. Activity was also seen in heart, kidneys, bladder and brain.

Quantitative analysis of the [^{18}F]FDG PET/CT images, showed that [^{18}F]FDG accumulation in the arthritis joints increases in concordance with the development of arthritis in both groups until day 0. After treatment with PLP-containing LCL, a strong reversal in joint uptake was seen at day 4, compared to the joint uptake at day 0. In mice that received empty LCL, joint uptake significantly increased at least for 7 days after induction of arthritis. No change in tissue uptake was seen in the internal control joints in time or after receiving PLP-containing LCL or empty LCL (Fig. 4E).

The knee joints were analyzed histologically to assess therapeutic effects on inflammatory activity and bone erosion of a single injection of 10 mg/kg PLP-containing LCL (Fig. 5). Histological examination showed that inflammatory activity in the synovium was reduced, although more abundant than in healthy joints, after PLP-LCL treatment (Fig. 5D). Additionally bone erosion was significantly lower in mice that received treatment with PLP-containing LCL compared with arthritic joints of untreated mice, but bone erosion was also present in healthy control joints.

4. Discussion

The present study demonstrates that [^{18}F]FDG PET/CT imaging can be used to visualize the progression of AIA in mice. Moreover, we showed that [^{18}F]FDG PET/CT imaging is a useful, non-invasive tool to monitor therapeutic effects of PLP-containing LCL in murine AIA. [^{18}F]FDG uptake in the inflamed joints, as a measure of inflammation severity, could also be determined quantitatively and reflected therapeutic efficacy non-invasively. Therapeutic effects were tracked closely by macroscopic scoring of the mice and by histological evaluation of affected joints. We were able to monitor the therapeutic effect of PLP-containing LCL with [^{18}F]FDG PET/CT imaging. Although macroscopically no swelling of the joints in treated mice was observed (Fig. 3), joint uptake of [^{18}F]FDG at initiation of treatment was shown (Fig. 4E, day 0). The tissue uptake in the contralateral joint remained the same. This indicates that even mild inflammation in the arthritic joint can be detected with [^{18}F]FDG PET/CT. Histology of the knee joints confirmed that

treatment with PLP-containing LCL suppressed the synovial inflammation. The suppressed inflammation also explains the inhibited bone erosion, since it is correlated with bone erosion and osteoclast activation [21].

Previous studies, which assessed the use of PLP-containing LCL as a treatment of experimental arthritis, reported a rapid, profound and more sustained effect of liposomal drugs, compared to administration of the free drugs. They showed that inflammation in treated animals was decreased and as a result, bone erosion reduced [11,13]. Others also evaluated the use of [^{18}F]FDG PET (or PET/CT) as a non-invasive tool to examine RA and reported that this method can be used to assess disease activity and response monitoring in experimental arthritis [15, 22,23]. Here, we have demonstrated in vivo quantification of progression of AIA and response monitoring after treatment with PLP-containing LCL, using [^{18}F]FDG PET/CT.

Since therapeutic effects were observed within 4 days after treatment and FDG uptake was assessed longitudinally, [^{18}F]FDG PET/CT imaging potentially is superior to conventional methods to score arthritis, such as macroscopic or histological scoring. Therapeutic effects were already seen macroscopically within one day after treatment, but this method is not quantitative as is [^{18}F]FDG PET/CT, while longitudinal assessment is not possible using histology.

The possibility to study the same animal at multiple time points during the progression of the disease or during therapy, can increase statistical power as a result of continues values, which would allow parametric testing. Additionally, the mice are kept alive to analyze all joints simultaneously and at different time points, which may take out some of the variation compared to histological assessments after dissection. Also extra information can be acquired, because the results that are

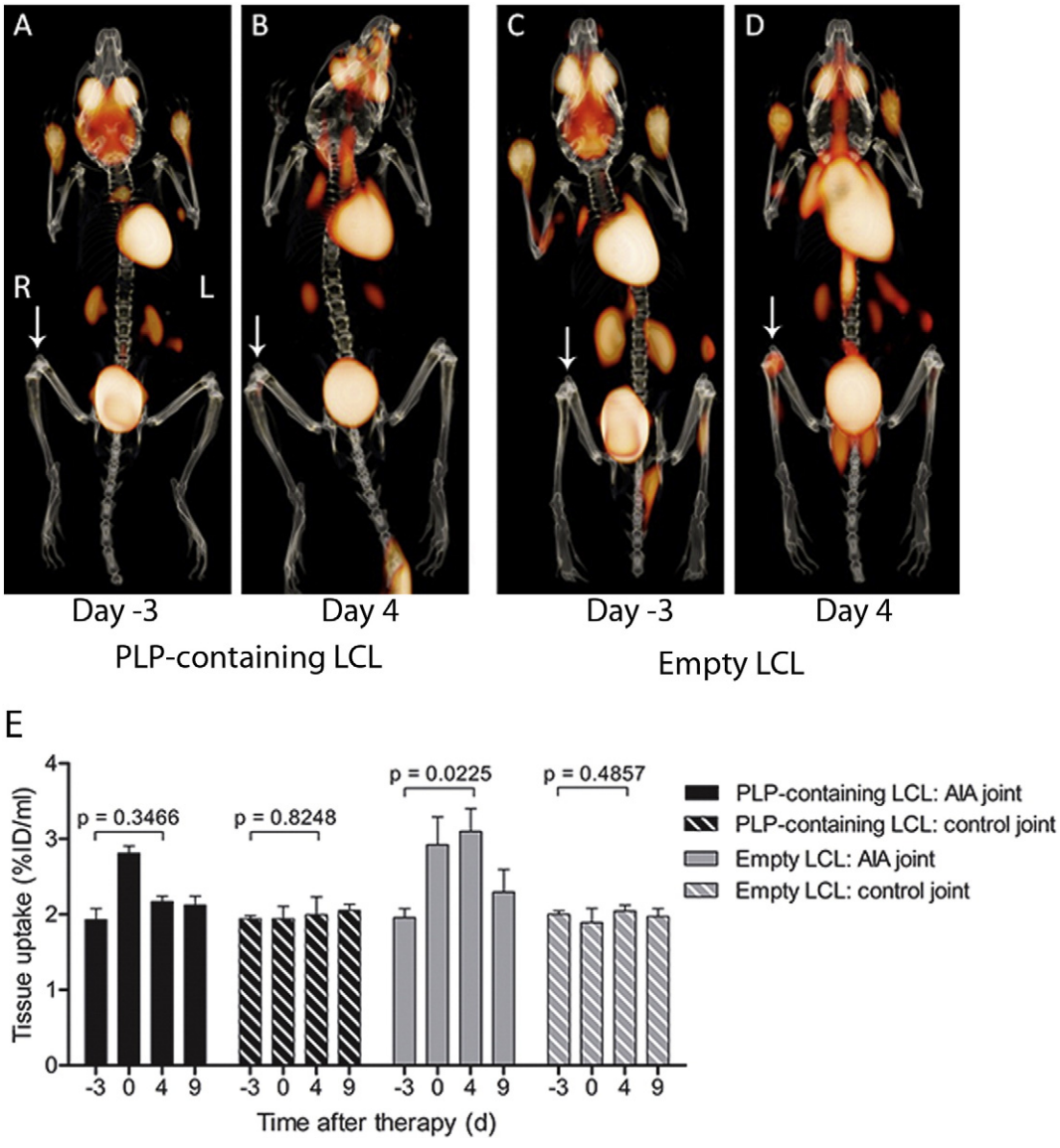

Fig. 4. [^{18}F]FDG PET/CT imaging (45 min p.i.) to visualize the [^{18}F]FDG accumulation in the course of AIA after treatment with 10 mg/kg PLP-containing LCL. Representative [^{18}F]FDG PET/CT images of a mouse that was treated with PLP-containing LCL (A–B) and a mouse that received empty LCL (C–D) at day −3 and day 4 after treatment are shown. Note that the [^{18}F]FDG accumulation in the front paws also decreased after treatment with PLP-containing LCL. Also activity was found in heart, kidneys, bladder and brain. Arrows indicate [^{18}F]FDG uptake in joints in which arthritis was induced. E: Tissue uptake (%ID/ml) in arthritic joints (right knee joint) and control joints (left knee joints) after treatment with PLP-containing LCL or empty LCL, 3 days after intra-articular induction of arthritis. Note that a strong reversal is seen after treatment with PLP-containing LCL and no significant difference in tissue uptake is observed compared to day −3 ($p = 0.3466$), while a significant increase in tissue uptake was found in mice that received empty LCL ($p = 0.0225$). The control joints of both groups did not show any difference between uptake after receiving therapy or Empty LCL ($p = 0.8248$ and $p = 0.4857$, respectively). Graph values represent mean ± SD, $n = 6$ mice per group. The significance of difference was tested by Mann–Whitney *U* test.

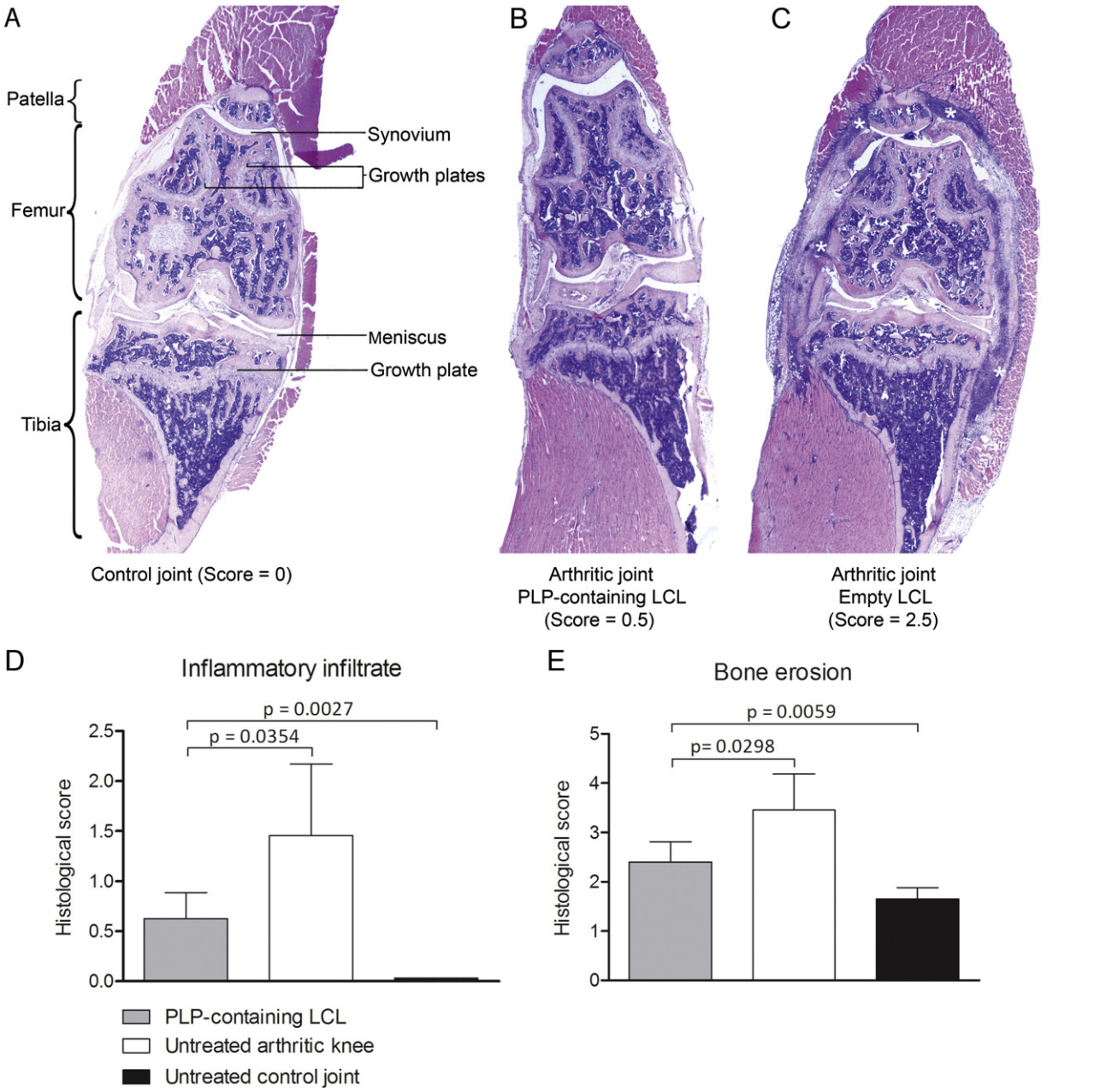

Fig. 5. Histological cross sections of whole knee joint from the control joint of an untreated mouse (A), of the arthritic knee of a mouse that received 10 mg/kg PLP-containing LCL (B), and of whole knee joint from the arthritic knee of a mouse that received empty LCL (C). Note the swelling and the abundant inflammatory infiltrate (stars) and that there is more inflammatory infiltrate in the untreated joint than in the joint of mice treated with PLP-containing LCL (B). D: Quantification of the synovial infiltrate. Inflammatory activity in arthritic joints was reduced after treatment with PLP-containing LCL ($p = 0.0354$), and no inflammation was observed in the untreated control joint ($p = 0.0027$). E: Quantification of Bone erosion of patella, femur and tibia. Bone erosion was suppressed after treatment with PLP-containing LCL ($p = 0.0298$), but, to a lesser extent, also present in healthy joints ($p = 0.0059$). Graph values represent mean ± SD, $n = 6$ mice per group. The significance of differences between groups was tested by Mann–Whitney *U* test.

obtained are not limited to joints or organs of interest. As shown in the present study, in which [^{18}F]FDG uptake in the front paws of the mice was observed, although not assessed as these joints were not analyzed histologically, this is most likely due to an inflammatory reaction against mBSA in CFA which was injected in the footpad during the immunization procedure. But also here a therapeutic effect of the treatment was found, emphasizing that i.v. administration of PLP-containing LCL leads to accumulation of PLP in multiple inflamed joints. Considering that [^{18}F]FDG accumulates in inflamed joints due to molecular changes, rather than due to anatomical changes, [^{18}F]FDG PET/CT imaging might also be able to detect early arthritis onset. Especially subclinical arthritis without symptoms. Potentially this could be used for the treatment in the earlier phases of exacerbations, which could prevent chronic effects, such as bone loss, and could suppress flare-ups.

It is of utmost importance to interpret [^{18}F]FDG uptake in inflamed joints using the baseline uptake as a reference (day 0 when monitoring the progression of the disease, or day −3 when monitoring the therapeutic effect of PLP-containing LCL). [^{18}F]FDG uptake (in terms of %ID/ml) in the mouse joints varied considerable, which hampers the analysis and the detection of changes in tissue uptake. [^{18}F]FDG cannot differentiate between arthritis or other processes with enhanced metabolic activity. To improve the use of non-invasive imaging tools in arthritis, other more specific tracers might be more valuable. Specific targeting of activated macrophages might increase the value of non-invasive

imaging methods. Other studies show already promising results with targeting of the folate receptor and TSPO, which are both overexpressed by activated macrophages found in RA [24,25]. The use of radiolabeled antibodies against other targets that are (over)expressed in RA, such as fibroblast activation protein or TNFα, might increase the specificity of the method [22]. As for now, these agents are not that well accepted in the clinic if compared to [^{18}F]FDG PET/CT.

5. Conclusions

We showed that treatment of arthritis with PLP-containing LCL reduced [^{18}F]FDG accumulation in arthritic joints, which was confirmed with suppression of joint swelling and decreased inflammatory activity and bone erosion. It showed that therapeutic effects can be monitored non-invasively with whole body [^{18}F]FDG PET/CT, even in an early stage after treatment initiation. This study shows the potential application of [^{18}F]FDG PET/CT for early liposomal drug response monitoring in RA.

Acknowledgments

We thank Bianca Lemmers-van de Weem, Henk Arnts, Iris Lamers-Elemans, and Kitty Lemmens-Hermans for their technical assistance in the animal experiments. This study was supported by a grant from the NanoNextNL Drug Delivery Programme (3D), project number 03D.06.

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