



Clinical pain research

Visualization of painful inflammation in patients with pain after traumatic ankle sprain using [¹¹C]-D-deprenyl PET/CT



Mikko Aarnio^{a,*}, Lieuwe Appel^{b,e}, Mats Fredrikson^{c,i}, Torsten Gordh^a, Olof Wolf^d, Jens Sörensen^{b,e}, Måns Thulin^f, Magnus Peterson^g, Clas Linnman^h

^a Department of Surgical Sciences, Anesthesiology and Intensive Care Medicine, Uppsala University Hospital, Sweden

^b PET Centre, Department of Medical Imaging, Uppsala University Hospital, Sweden

^c Department of Psychology, Uppsala University, Sweden

^d Department of Surgical Sciences, Orthopedics, Uppsala University Hospital, Sweden

^e Section of Nuclear Medicine and PET, Department of Surgical Sciences, Uppsala University, Sweden

^f Department of Statistics, Uppsala University, Sweden

^g Department of Public Health and Caring Sciences, Section of Family Medicine and Preventive Medicine, Uppsala University, Sweden

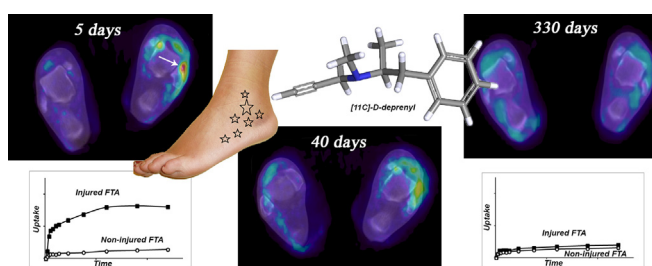
^h Department of Anesthesiology, Boston Children's Hospital, Harvard Medical School, Boston, MA, USA

ⁱ Department of Neuroscience, Karolinska Institutet, Stockholm, Sweden

HIGHLIGHTS

- An increased [¹¹C]-D-deprenyl uptake is shown in painful locations after ankle sprain.
- Patients experiencing persistent pain had prolonged peripheral D-deprenyl uptake.
- The described method can visualize, quantify and follow pain generating processes.
- Such an objective correlate may represent a progress in pain research and management.

GRAPHICAL ABSTRACT



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ABSTRACT

Background and aims: Positron emission tomography (PET) with the radioligand [¹¹C]-D-deprenyl has shown increased signal at location of pain in patients with rheumatoid arthritis and chronic whiplash injury. The binding site of [¹¹C]-D-deprenyl in peripheral tissues is suggested to be mitochondrial monoamine oxidase in cells engaged in post-traumatic inflammation and tissue repair processes. The association between [¹¹C]-D-deprenyl uptake and the transition from acute to chronic pain remain unknown. Further imaging studies of musculoskeletal pain at the molecular level would benefit from establishing a clinical model in a common and well-defined injury in otherwise healthy and drug-naïve subjects. The aim of this study was to investigate if [¹¹C]-D-deprenyl uptake would be acutely elevated in unilateral ankle sprain and if tracer uptake would be reduced as a function of healing, and correlated with pain localizations and pain experience.

Methods: Eight otherwise healthy patients with unilateral ankle sprain were recruited at the emergency department. All underwent [¹¹C]-D-deprenyl PET/CT in the acute phase, at one month and 6–14 months after injury.

Abbreviations: PET, positron emission tomography; PET/CT, positron emission tomography/computed tomography; MAO, monoamine oxidase; [¹¹C]DDE, [¹¹C]-D-deprenyl; ACP, acute phase; FU1, first follow-up; FU2, second follow-up; VAS, visual analogue scale; SUV, standardized uptake value; ROI, region of interest; VOL, volume of interest; TACT, time activity; FTA, anterior fibulotalar ligament.

* Corresponding author at: Department of Surgical Sciences, Anesthesiology and Intensive Care Medicine, Uppsala University Hospital, ing 70 1 tr, 75185 Uppsala, Sweden.

E-mail address: mikko.aarnio@surgsci.uu.se (M. Aarnio).

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Results: Acute [^{11}C]-D-deprenyl uptake at the injury site was a factor of 10.7 (range 2.9–37.3) higher than the intact ankle. During healing, [^{11}C]-D-deprenyl uptake decreased, but did not normalize until after 11 months. Patients experiencing persistent pain had prolonged [^{11}C]-D-deprenyl uptake in painful locations.

Conclusions and implications: The data provide further support that [^{11}C]-D-deprenyl PET can visualize, quantify and follow processes in peripheral tissue that may relate to soft tissue injuries, inflammation and associated nociceptive signaling. Such an objective correlate would represent a progress in pain research, as well as in clinical pain diagnostics and management.

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1. Introduction

Pain is a uniquely personal experience, and self-reports remain the gold standard of assessment. However, self-reports are affected by numerous factors [1]. Objective visualization and quantification of peripheral musculoskeletal injury that generates nociceptive input would represent a progress in pain research. The visualization of pain-associated processes in peripheral tissue would facilitate diagnosis, strengthen patients' self-report and aid clinical decisions. Further quantification of nociception related processes in peripheral tissue would guide clinical assessment and treatment monitoring.

Positron emission tomography (PET) is increasingly used to diagnose, characterize and monitor disease activity in peripheral inflammatory disorders of both known and unknown origin [2,3]. Nociceptive pain is a cardinal feature of inflammation, and although tissue injury that induces inflammation plays a pivotal role in the generation of pain [4], few studies have localized and quantified the extent of peripheral injury and inflammation in pain patients with molecular imaging.

[^{11}C]-D-deprenyl (D-[N-methyl- ^{11}C]N, N-methyl, propargylamphetamine) is the D-isomer of deprenyl, a well-characterized monoamine oxidase-B (MAO-B) inhibitor with anti-inflammatory and neuro-protective properties. Based on the available data D-deprenyl binds to MAO enzymes located in the outer mitochondrial membrane of cells engaged in processes involved in inflammation and repair [5]. Previous PET studies have shown high and irreversible [^{11}C]-D-deprenyl ([^{11}C]DDE) uptake in synovial inflammation of knees in rheumatoid arthritis patients [6] and in patients with chronic whiplash associated disorder [^{11}C]DDE uptake was increased in the painful neck regions [7]. These studies strongly suggest an association of [^{11}C]DDE uptake in soft tissue injury and inflammation with associated pain. However, it is not known if [^{11}C]DDE PET can indicate acute tissue damage and pain, and if the healing process can be followed over time in the setting of acute injury. The purpose of this study was to verify and extend previous [^{11}C]DDE-PET findings by investigating tracer retention in unilateral ankle sprain. Traumatic ankle sprain presents a suitable model with substantial pain in the acute phase and reasonably rapid resolution of pain within months. With isolated traumatic ankle sprain, the intact ankle can be used as a within-subject non-affected control. The hypotheses were that [^{11}C]DDE uptake would be acutely elevated localized to injured tissues, reduced as a function of healing, and correlated with subjective pain localizations and pain experience.

2. Materials and methods

2.1. Patients and study design

Eight non-smoking patients with an acute isolated traumatic ankle sprain, diagnosed by an orthopedic surgeon, were recruited at the emergency department at Uppsala University Hospital,

Uppsala, Sweden. No patient had a history of a somatic or psychiatric disease or drug abuse.

The study design consisted of three [^{11}C]DDE PET/CT investigations on both ankles of each patient. The first imaging occasion (acute phase; ACP) was scheduled within 5 days after the injury. The first follow-up imaging occasion (FU1) was scheduled 6 ± 1 weeks after the injury in order to monitor the subacute or repair phase of the tissue injury. The second follow-up (FU2) was scheduled 6 ± 1 months after the injury in order to monitor the remodeling phase of the tissue injury. During the course of the study it was noticed that complete healing and recovery of the injured peripheral tissue might take longer time than expected. Therefore, three of the patients had their FU2 imaging between 9 and 14 months after the injury. Before each PET/CT investigation, all patients refrained from analgesics and anti-inflammatory drugs for 24 h, from alcohol and caffeine for 12 h, and from food for 3 h.

2.2. Pain assessment

Immediately before and after PET/CT investigations, patients rated their average resting pain levels in the feet on a visual analogue scale (VAS), ranging from 0 (no pain) to 10 (worst imaginable pain). The locations of maximal tenderness were palpated and marked on an anatomical picture of the foot. The patient characteristics are presented in Table 1.

2.3. PET/CT scanning

The radioligand [^{11}C]DDE was produced at the chemistry section of the Uppsala PET center according to a standard manufacturing procedure with previously published methods [8,9]. All patients were investigated with a GE Discovery ST PET/CT scanner (General Electric Medical Systems, Milwaukee, WI, USA). The scanner provides 47 contiguous planes of data with a distance of 3.27 mm and 3.125 mm transaxial and axial resolution: this gives a total axial field of 15.7 cm. Patients were scanned in the supine position with the feet gently supported by a vacuum cushion to minimize foot and ankle movement. The PET/CT investigation was initiated with a short CT scan (140 kV; auto mA 10–80 mA) for attenuation correction of the PET emission data. Each patient received an intravenous bolus of approximately 5 MBq/kg [^{11}C]DDE in an antecubital vein. Simultaneously, a dynamic emission scan (3D mode) was initiated with a predetermined set of measurements (frames of 4×30 ; 3×60 ; 2×300 , and 3×600 s) for up to 45 min. PET data were reconstructed with the OSEM (Ordered Subset Expectation Maximization) algorithm with 2 iterations and 21 subsets and with a 2.57 mm wide post-processing filter. PET data were corrected for decay, photon attenuation, scatter, random coincidences and dead time.

Table 1
Demographic and clinical characteristics of the included patients.

Case	Gender ^a	Age	Cause of injury	Day of investigation ^b			VAS at rest ^c		
				ACP	FU1	FU2	ACP	FU1	FU2
1	M	31	Inversion in football	4	37	194	2	0	0
2	F	21	Inversion in stairs	2	35	420	1	0	0
3	M	28	Inversion in floorball	5	40	336	3	3	0
4	M	32	Fall from ladders	2	36	163	1	0	3
5	F	20	Inversion in football	9	65	161	1	0	4
6	F	50	Fall from chair	2	37	210	6	0	0
7	M	38	Inversion after jump	3	44	174	8	0	0
8	F	58	Inversion when walking	2	37	262	4	2	0

^a M = male, F = female.

^b Days from ankle sprain injury, where ACP is the investigation in the acute phase, and FU1 and FU2 are the first and second follow-up investigations.

^c Visual analogue score (VAS), ranging from 0 (no pain) to 10 (worst imaginable pain).

2.4. Image analysis

The image analysis was performed with VOIager (version 2.0.5; GE Healthcare 2009). A summation image (25–45 min post tracer injection) was used for delineation of regions of interest (ROI). First, in each imaging occasion the lesions were identified visually from the area with elevated [¹¹C]DDE uptake above the background signal. Second, an irregular ROI was delineated manually by drawing an isocontour that was defined as 50% of the maximum voxel signal intensity within the lesion. Isocontours were outlined in multiple adjacent axial slices showing tracer uptake within the target lesion resulting in a polygonal volume of interest (VOI) for each defined region. The superimposed CT image was used to delineate anatomically corresponding reference regions of the same size in the contralateral foot. For images from the other two occasions, the same regions as in the original lesion were defined from residual [¹¹C]DDE uptake, and if there was no elevated [¹¹C]DDE uptake, VOIs were delineated and positioned anatomically using the CT images. The defined regions of interest were checked, identified and named in consultation with an experienced PET radiologist. Because of the limits of spatial resolution in PET/CT, the defined anatomical locations were approximate, corresponding to the nearest identifiable region in superimposed CT image and may also have included surrounding tissue.

2.5. Data analysis

Time activity (TACT) data were generated for all scans from each patient: this represented the dynamic sequence of radioactivity levels for the VOI in each PET/CT scan from 0 to 45 min. The TACT data were standardized with respect to the administered dose of radioactivity and the patient's body weight, resulting in a standardized uptake value (SUV).

For the generation of SUV-related parameters, the data from 25 to 45 min post tracer administration was used to limit the effects of blood flow. In the summation image the mean and maximum voxel value were derived for each VOI (SUV_{MEAN} and SUV_{MAX}, respectively). Subsequently, SUV_{RATIO} was computed for each VOI as the ratio of SUV_{MEAN} between the injured foot and the non-injured foot.

2.6. Statistical analysis

SUV_{MEAN}, SUV_{MAX} and SUV_{RATIO} were evaluated within and between scans for each patient. Differences in SUV_{MEAN}, SUV_{MAX} and SUV_{RATIO} were evaluated with Wilcoxon's matched pairs test. Correlations between SUV_{MEAN}, SUV_{MAX} and SUV_{RATIO} and subjective pain ratings were assessed with Spearman's Rank Order test. Unless otherwise stated, results are presented as median (range) values.

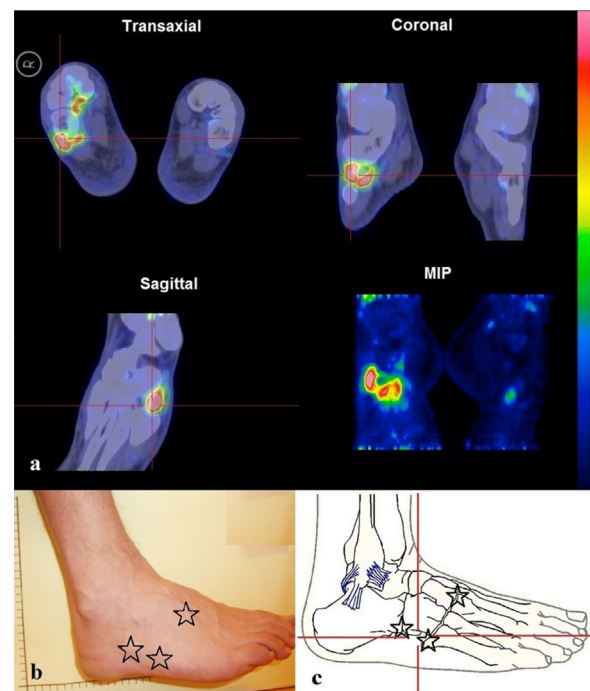


Fig. 1. Patient case illustrating the acute phase. Regions of elevated [¹¹C]-D-deprenyl uptake corresponded with clinical findings and painful locations.

A 31-year-old male (case 1) four days after an inversion injury in the right mid-foot. (a) Fused [¹¹C]DDE PET/CT images of the feet of (highest value in purple). The images illustrate the plantar region of uptake between cuneiform and 1st, 2nd and 3rd metatarsal bones. The crosshair points to elevated [¹¹C]DDE uptake in the calcaneocuboid joint and regions between cuboideum and 5th metatarsal bone. (b) A photograph of the same right foot with the points of maximal tenderness (stars) corresponding to the regions of high [¹¹C]DDE uptake. (c) An anatomical image of the right foot with the tender points and crosshair in corresponding locations. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

Subjective pain rating and elevated uptakes were also dichotomized (to presence/non-presence) for both feet for all patients at all three investigations. The dichotomized data was analyzed using a mixed logistic regression model [10], using pain as the response variable and elevated uptake, patient ID and investigation number as explanatory variables.

3. Results

3.1. Clinical and radiographic results

The most common mechanism of injury was lateral ankle inversion sprain present in five out of eight patients. There were no

injuries to the bone structure or dislocations observed on plain radiographs or from computed tomography in any of the patients. Pain at rest (VAS) decreased markedly from 2.5 (1–8) at ACP to 0 (0–3) at FU1 ($p=0.011$). VAS ratings did not change significantly between FU1 and FU2 ($p=0.890$). See also Table 1. At ACP, no patients could support themselves completely on the injured foot and they reported intense pain during weight bearing. At FU1 and FU2, all eight patients still experienced pain with extreme ankle joint movements or pain during heavy weight bearing on the injured foot, however, this pain was less intense than at ACP.

3.2. PET/CT results

At ACP an elevated regional [^{11}C]DDE uptake was observed in the injured foot in all patients, with only a very low tracer uptake present in the non-injured foot. The regions of elevated tracer uptake corresponded with clinical findings and painful locations. A typical patient is presented in Fig. 1.

In five out of eight cases, the most common locations for increased signal intensity were the anterior talofibular ligament, the calcaneofibular ligament and parts of the deltoid ligament (Table 2).

The SUV_{MEAN} of the lesions was 1.9 (1.2–3.1) at ACP, 1.7 (0.4–3.0) at FU1, and 0.8 (0.2–1.8) at FU2. In the corresponding anatomical locations in the non-injured foot, the SUV_{MEAN} was similar at each imaging occasion: 0.3 (0.1–1.1), 0.3 (0.1–0.6) and 0.3 (0.1–0.6) at ACP, FU1 and FU2, respectively.

The SUV_{MAX} of the lesions was similar at ACP and FU1: 2.8 (1.8–5.1) at ACP and 2.8 (0.7–4.7) at FU1. However, the SUV_{MAX} was substantially smaller 1.2 (0.4–3.2) at FU2. The decrease in SUV_{MAX} between ACP and FU2 was 57% ($p=0.012$). In the corresponding anatomical locations in the non-injured foot, SUV_{MAX} was similar at each imaging occasion: 0.5 (0.1–1.2), 0.6 (0.1–1.0) and 0.6 (0.2–1.1) at ACP, FU1 and FU2, respectively.

The $\text{SUV}_{\text{RATIO}}$ showed that the regional [^{11}C]DDE uptake in the injured locations was initially 10.7 (2.9–37.3) times higher than tracer uptake in the corresponding regions of the non-injured foot. The $\text{SUV}_{\text{RATIO}}$ reduced to 5.6 (2.5–8.3) at FU1 and 2.8 (0.7–3.8) at FU2. [^{11}C]DDE uptake decreased by 48% ($p=0.017$) between ACP and FU1, and by 50% ($p=0.012$) between FU1 and FU2. The decrease between ACP and FU2 was 74% ($p=0.017$). A typical patient illustrating these findings is presented in Fig. 2.

At later stages (FU1 and FU2), additional regions of elevated [^{11}C]DDE uptake were observed in the injured foot and even in the non-injured foot. Two patients (cases 5 and 7) had elevated [^{11}C]DDE uptake in the non-injured foot, that was also noted at ACP (Table 3).

The correlation between FU1 $\text{SUV}_{\text{RATIO}}$ and FU2 VAS pain score was $r=0.764$ ($p=0.03$). No other correlations between self reported pain intensity at rest (VAS) and SUV values were present at ACP, FU1 or FU2. However, dichotomized pain was significantly associated with dichotomized elevated uptake ($p=0.036$). The estimated odds ratio for presence of pain for feet with elevated uptake compared to feet with no elevated uptake was $\text{OR}=5.06$ (Table 4).

4. Discussion

Accumulation of [^{11}C]DDE uptake in the injured tissue was evident in painful locations in all patients after injury, confirming the initial hypothesis. The relatively high regional [^{11}C]DDE uptake, compared to surrounding structures, was localized in the regions of the ligaments and joints and was concurrent with the clinical examination and the mechanism of injury. The uptake of [^{11}C]DDE tracked the normal recovery process of acute traumatic ankle sprains. The tracer uptake was still elevated several months

after initial injury, and patients with persistent pain had prolonged [^{11}C]DDE uptake at the injury site.

4.1. Characteristics of [^{11}C]DDE uptake

The ratio of uptake between the injured and the reference regions provided the best demonstration of high initial [^{11}C]DDE uptake and a recovery-associated decrease. The SUV_{MAX} value was less sensitive, but this value is less dependent on the placement and drawing of ROI and is not as reproducible as the SUV_{MEAN} value [11,12]. Because kinetic modeling of tracer uptake has many unknowns, we opted to base all analyses on data obtained 25–45 min post tracer injection. Although SUV may be an oversimplification of physiological events, and as a quantitative index, is vulnerable to several sources of variability [13–15], SUV was used because of its simplicity, blood sampling is unnecessary, and because it is well suited for exploratory follow-up with a control region. During follow-up, the SUV values in the control regions were stable; indicating the reliability of SUV_{MEAN} and SUV_{MAX} was good.

The localization of the tracer uptake correlated with the expected anatomical localization in acute ankle sprains. The anterior talofibular ligament is commonly injured in a lateral ankle sprain due to its low load tolerance and anatomical position. The tracer uptake in the mid-foot ligaments was expected [16], although the high amount of uptake near the medial joint area or the deltoid ligament was a new finding.

The normal recovery process [17] was reflected by serial PET imaging. New regions of increased uptake that represented new tender points in the injured foot were evident over time. The time needed for completion of healing and recovery of ankle sprains varies and the incidence of residual complaints is high years after injury [16–18]. Increased [^{11}C]DDE uptake was evident several months after initial injury. The new regions of increased tracer uptake in the injured foot in some cases might be the result of abnormal kinematics with ligament insufficiency and the first signs of degenerative and/or adaptive changes [19]. Ligament instability alters the contact mechanics in the joint and causes wear and increased shear loading along with inflammatory processes [20,21]. Some patients could have suffered slight re-injury as this is common after ankle sprains without recalling or reporting it. The same explanations could account for some regions with high initial tracer uptake and no decrease during follow-up. Increased loading and overuse of the non-injured foot, with increased stress to the joints and bone surfaces, might cause the observed elevation in tracer uptake on the non-injured side.

4.2. Pain and [^{11}C]DDE uptake

Pain while weight bearing and pain during extreme movements of the ankle joint coexisted with increased [^{11}C]DDE uptake. The odds of a patient having pain was more than 5 times higher given an elevated uptake compared to no elevated uptake. In line with Table 4, this means that 30% of all patients have pain in feet with no elevated uptake, 68% of all patients will have pain in feet with elevated uptake. Although $\text{SUV}_{\text{RATIO}}$ at first follow-up was positively correlated with pain at last follow-up, there were no other correlations between pain intensity (VAS-score) at rest and the SUV-related measures. There may be a relationship between [^{11}C]DDE uptake and subsequent risk of persisting pain following trauma. This is reasonable if the initial [^{11}C]DDE uptake is associated with severity of injury [22]. However, in a large seven-year follow-up of 648 patients with sprained ankles, there was no correlation between initial severity of the sprain and residual disability at follow-up [16]. The perception of pain is not a simple linear phenomenon, and nociceptive input can be modulated at several levels of the pain pathway, which would attenuate the direct influence of

Table 2
Regions of the most elevated [¹¹C]-D-deprenyl-uptake. Standardized uptake values (SUV) and the ratio between injured and non-injured foot are given for the acute phase and follow-up investigations.

Case	Anatomical location ^a	SUV _{MAX} ^b			SUV _{RATIO}		
		ACP	FU1	FU2	ACP	FU1	FU2
1	Calcaneocuboid joint R	4.9	5.2	2.9	2.9	2.0	2.2
	Plantar tarsometatarsal ligaments/tarsometatarsal joint between cuboideum and 5th metatarsal R	3.8	3.2	4.2	4.2	3.4	5.1
	Plantar tarsometatarsal ligaments/tarsometatarsal joints between cuneiform and 1st, 2nd and 3rd metatarsal R	3.0	4.5	1.8	2.2	5.5	2.2
2	Anterior talofibular ligament (FTA) and calcaneofibular ligament (FC) L	2.4	1.9	0.8	6.6	4.3	1.4
	Talar neck and anterior tibiotalar part of deltoideus L	2.7	1.6	0.7	6.6	4.8	1.0
	Tibiocalcaneal part of deltoid ligament L	2.4	1.7	0.7	5.5	4.3	1.0
	Dorsal calcaneocuboid ligament/calcaneocuboid joint L	3.0	2.1	0.7	8.8	4.3	1.3
	1st Cuneiform bone (medial. insertion of tendon tibialis anterior) L	3.1	0.9	0.4	9.7	2.9	1.4
3	Anterior talofibular ligament (FTA) L	2.7	1.8	0.7	6.0	2.4	1.3
	Medial aspects of talocrural joint L	1.8	1.0	0.4	7.7	5.3	0.7
	Posterior talofibular ligament (FTP) L	1.7	1.2	0.4	4.6	2.5	0.7
4	Calcaneofibular ligament (FC) R	5.1	4.2	3.2	15.2	8.0	3.3
	Space between the calcaneus and the Achilles tendon R	5.2	4.7	2.9	17.3	7.4	2.4
	Ventrally from medial malleolus and navicular bone/talonavicular ligament R	3.7	4.9	3.6	10.6	7.8	6.3
5	Anterior talofibular ligament (FTA) L	2.3	5.2	2.4	8.0	9.7	2.6
	Posterior tibiotalar part of deltoid ligament L	2.4	3.8	2.1	14.8	6.4	7.8
	Insertion of the calcaneofibular ligament (FC) L	2.5	4.3	1.6	20.1	8.3	3.8
6	Interosseus talocalcaneal ligament/sinus tarsi L	2.4	1.2	0.3	38.4	27.1	1.1
	Sustentaculum tali L	3.5	0.8	2.2	44.1	9.9	6.9
	Anterior tibiotalar part of the deltoid ligament L	2.9	0.5	0.2	29.0	4.6	1.1
	Medial process of calcaneus L	2.8	0.6	0.6	36.1	4.9	4.3
7	Anterior and posterior parts of the deltoid ligament L	3.3	3.2	1.0	11.3	2.8	2.4
	Calcaneofibular ligament (FC) L	3.3	4.4	0.4	11.5	8.4	1.2
	Anterior talofibular ligament (FTA) S. interosseus talocalcaneal ligament (IOL) L	3.2	3.4	0.9	8.7	4.6	1.8
	Dorsal parts of navicular bone L	2.2	3.5	0.9	12.4	6.9	2.6
	Short plantar ligament	2.4	2.7	0.3	13.3	3.1	0.7
8	Medial malleolus surface of the talus R	2.8	2.0	1.3	12.8	5.1	2.0
	Calcaneofibular ligament (FC) R	3.1	2.2	1.2	11.6	7.3	1.6
	Interosseus talocalcaneal ligament (IOL) R	2.7	1.9	1.6	11.6	5.8	3.7
	Anterior tibiofibular ligament (FTA) R	2.3	2.3	1.6	7.9	7.7	3.7
	Talonavicular ligament R	2.0	1.9	1.5	6.8	5.8	3.0
	Anterior tibiotalar part of the deltoid ligament R	2.1	2.7	2.5	8.4	8.6	4.8

^a L = left side, R = right side.

^b ACP, FU1 and FU2 represent the acute phase, first follow up and second follow-up.

Anatomical locations are approximate, corresponding to the nearest identifiable region and may also include surrounding tissue.

Table 3
New regions with elevated [¹¹C]-D-deprenyl-uptake in the injured and the non-injured foot during the follow-up investigations.

Case	Anatomical location	SUV _{MAX}			SUV _{RATIO}		
		ACP	FU1	FU2	ACP	FU1	FU2
1	Subtalar joint (posterior part) L	1.1	2.6	2.5	0.4	1.5	2.4
	Origin of calcaneofibular ligament (FC) L	1.0	1.9	3.0	0.5	1.3	1.9
	Sustentaculum tali L	0.5	1.4	2.3	0.9	1.3	1.0
4	Calcaneus (posterior and lateral parts) L	0.2	0.4	5.4	0.1	0.1	3.1
	Talocrural joint/trochlear tali R	2.2	5.2	4.6	7.0	17.0	4.3
5	Calcaneus (posterior and lateral parts) R	0.2	0.4	2.4	0.2	0.4	4.7
	Subtalar joint (middle and anterior articulation) and sinus tarsi R	4.7	4.2	4.6	10.5	10.9	3.6
	Dorsal calcaneocuboid ligament/calcaneocuboid joint L	0.6	1.3	2.8	3.3	2.0	6.8
	Anterior part of ankle joint capsule L	1.8	4.0	4.7	5.0	5.6	13.2
	Posterior tibiotalar part of the deltoid ligament L	1.6	4.0	2.3	5.3	5.6	4.5
6	Ligament/joint between cuboideum and 5th metatarsal R	0.9	1.5	1.1	4.1	19.0	13.1
	Dorsal parts of the neck of talus L	0.1	0.2	0.8	0.1	0.9	3.3
	Dorsal tarsometatarsal joint between ACP cuneiform and ACP metatarsal L	0.1	0.0	0.9	0.6	1.0	6.2
	Sulcus calcanei L	0.4	0.1	0.7	5.4	1.2	4.0
	Plantar calcaneonavicular ligament L	1.1	0.3	0.8	6.3	1.1	3.9
7	Origin of posterior tibiotalar part of deltoid ligament R	2.0	2.7	1.3	7.3	3.2	3.2
	Flexor digitorum brevis muscle R	0.4	0.5	1.3	1.1	0.9	3.1
8	Sustentaculum tali L	1.3	1.9	2.8	2.8	3.8	6.5

Explanations as in Table 2.

Anatomical locations in the non-injured foot are shown in bold.

Anatomical locations presented in cursive were visually detected in the acute phase.

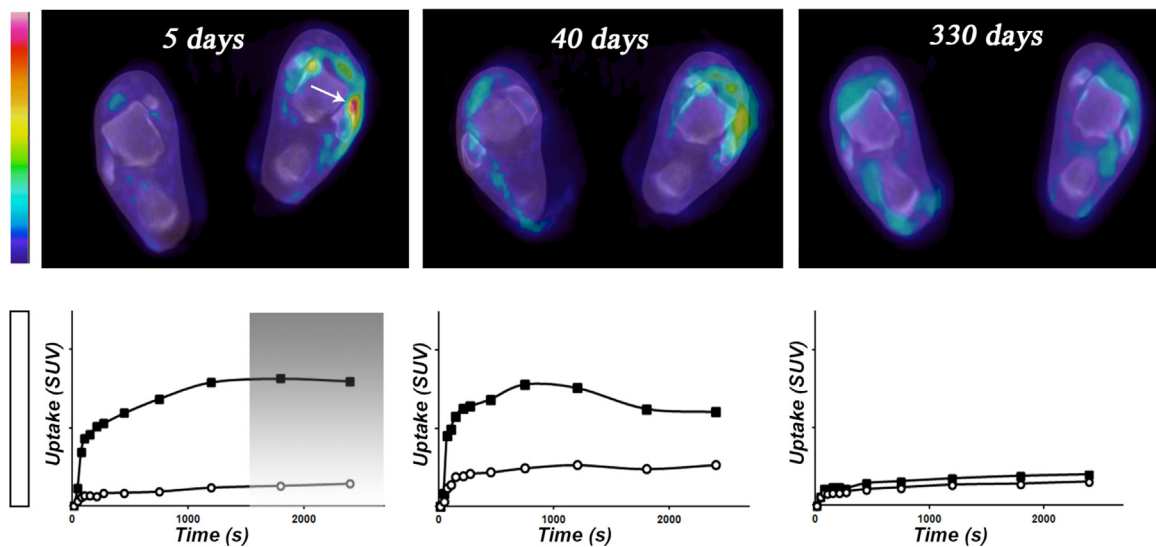


Fig. 2. Patient case illustrating decreased [^{11}C]DDE uptake between acute injury and follow-up.

A 20-year-old female (case 3) with a classic lateral inversion ankle sprain. The top row displays fused transaxial [^{11}C]DDE PET/CT images of the feet. The color bar indicates SUV values from 0 (dark blue) to 2.5 (red). Location of left anterior fibulotalar ligament (FTA) is indicated by a white arrow. The bottom row displays respective time activity (TACT) curves (black rectangles represent injured FTA and white circles control region). The bar indicates SUV_{MEAN} values from 0 (bottom) to 2.5 (top). The gray rectangle indicates the data (25–45 min post tracer administration) that was used for the generation of dependent variables (SUV_{MEAN}, SUV_{MAX} and SUV_{RATIO}). The region of FTA initially showed visually significant [^{11}C]DDE uptake that was undetectable 11 months after the injury indicating recovery of the tissue damage. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

Table 4

Pain and elevated [^{11}C]DDE uptake.

	Elevated uptake ^a	No elevated uptake	Total (%)
Pain ^b	26 (54%)	3 (6)	29 (60)
No pain	12 (25)	7 (15)	19 (40)
Total (%)	38 (79)	10 (21)	48 (100)

^a SUV_{MAX} > 1.2 and/or SUV_{RATIO} > 2.0.

^b pain while weight bearing or in extreme motions (yes/no).

the primary processes at the injury site. Subjective pain estimates were measured at rest without any provocation or loading of the ankle joint or attempt to measure mechanical ankle laxity or instability. It is difficult to standardize such a provocation, but more rigorous pain analysis and a measurement of disability are needed in future studies to address the link between peripheral pain induction on the molecular level and subjective pain experience.

4.3. The limitations of the study and [^{11}C]DDE uptake mechanism

The limitations of this study are the small sample size and that the exact mechanism of the observed [^{11}C]DDE uptake is not fully known. The small sample size gives us lesser statistical power to detect differences. However, we believe that the sample size is appropriate for PET pilot study to describe and assess feasibility of using [^{11}C]DDE PET/CT in visualization of peripheral inflammation in musculoskeletal injury. D-deprenyl uptake appeared to take place mostly during the inflammatory phase of injury repair but was still noticeable in the proliferative and remodeling phases. Available data suggest that the major candidate for the binding site for D-deprenyl is mitochondrial MAO inside cells engaged in processes involved in inflammation and repair [5,23]. The role of MAO in inflammation is not fully studied. However, it has been shown that the MAO activity of human granulocytes and lymphocytes is predominantly of the B form [24] but in inflammation, MAO-A is one of the signature markers that is modified by interleukins and the expression of enzymes is strongly upregulated in activated monocytes and macrophages [25]. Deprenyl was originally synthesized as a “psychic energizer” through combining amphetamine

and pargyline, a MAO inhibitor, in the same molecule. D-deprenyl is a more potent inhibitor of MAO-A but it is up to 25–500 times less potent as an MAO-B inhibitor than its mirror isomer L-deprenyl [26–28] and therefore D-deprenyl has been utilized as a MAO-B inactive control tracer for brain studies [8]. Trapping mechanisms, other than binding to a specific molecular structure, are possible at the site of injury or inflammation. As the [^{11}C]DDE molecule is a weak lipophilic base, non-specific trapping mechanisms could include elevated blood flow, increased vascular permeability and enhanced transudation at the injury site. Full kinetic modeling of tracer uptake had been necessary to fully exclude blood flow components of tracer uptake. However, the exact mechanism of peripheral [^{11}C]DDE trapping was not known, so we did not obtain arterial blood sampling for patient safety concerns. In the dynamic PET, the rapid increase in the first frames was followed by a slight washout or plateau being present in the last frames, suggesting a mechanism of irreversible or slowly reversible tracer trapping. The shape of time activity curves indicates that enhanced blood flow is unlikely sole mechanism of tracer uptake. However, further studies are needed to elucidate the exact uptake mechanism of D-deprenyl and to characterize what parts of the injury-inflammation cascade are visualized by [^{11}C]DDE PET.

5. Conclusions

The present investigation demonstrates that molecular aspects of tissue lesions presenting with inflammation and pain may be visualized, quantified, and followed over time with PET/CT with [^{11}C]DDE being a promising tracer. [^{11}C]DDE may be valuable for identifying patients with problematic healing and prolonged inflammation and who are at high risk for developing persisting pain.

6. Implications

This relatively noninvasive technique represents an objective finding indicating processes in tissue injury from which nociceptive signals originate. Such an objective correlate would represent

a progress in pain research, as well as in clinical pain diagnostics and management.

Ethical issues

The study was approved by the Ethics Committee of the Faculty of Medicine at Uppsala University, Sweden, and by the Radiation Ethics and Safety Committee of Uppsala University Hospital, Uppsala, Sweden. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study.

Conflicts of interest

All authors declare that they have no conflicts of interest.

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