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Quantitative tracking of inflammatory activity at the peak and trough plasma levels of tofacitinib, a Janus kinase inhibitor, via in vivo ^{18}F-FDG PET

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Abstract

Purpose: To assess the capability of in vivo positron emission tomography (PET) using ^{18}F-fluorodeoxyglucose (^{18}F-FDG) to quantify changes in inflammatory activity in response to tofacitinib, a Janus kinase (JAK) inhibitor, over a timeframe of a few hours to few days in a preclinical model of rheumatoid arthritis (RA).

Methods: Twenty-four mice with collagen-induced arthritis in the following groups were assessed: Group 1, where the changes in PET measures for the extremity joints were evaluated at the peak and trough plasma drug levels after administration of a single dose of tofacitinib (4 hours apart); Group 2, where joint PET measures were assessed before treatment and after 6 days of administration of a daily dose of tofacitinib; and group 3 (controls), where joint PET measures were derived from the same mice, 6 days apart.

Results: At about peak plasma levels of the drug after a single tofacitinib administration, there was a reduction in PET measures compared to pretreatment values, suggesting decreased inflammatory activity. These measures were equivalent to those obtained after 6 days of daily dosing by tofacitinib. However, PET measures at trough plasma levels of the drug from tofacitinib administration were significantly higher than those at peak plasma drug levels and equivalent to pretreatment measures. There were insignificant changes in PET measures for the control animals.

Conclusion: ^{18}F-FDG PET can detect changes in inflammatory activity occurring in response to the JAK inhibitor tofacitinib: (a) during peak and trough plasma drug levels, that is within mere hours of treatment; and (b) over a span of days.

1 | INTRODUCTION

Joint inflammation is considered to be the hallmark of autoimmune arthritic conditions such as rheumatoid arthritis (RA). Therefore, a number of therapeutic agents, such as tumor necrosis factor-alpha antagonists[1–3] and Janus kinase inhibitors[4,5] have been developed to treat RA-associated joint inflammation, and several new treatments are at various stages of development.[4–6] It is critical to identify the treatment regimen which RA patients respond positively to, as this greatly improves their short- and long-term prognosis[7] and makes remission a realistic goal.[8] In this regard, the collagen-induced arthritis (CIA) mouse model has a long history of being useful in RA therapy research[9,10]; mice with CIA present with signs and pathology that mimic human RA, such as joint erythema and swelling, proliferative synovitis and pannus.[11,12] Unfortunately, visual assessment-based scoring of joint swelling commonly used for assessing RA treatment response lacks the sensitivity to detect therapeutic effects early. Tissue histology, the current gold standard for measuring RA disease activity, requires sacrificing the animal, therefore precluding the opportunity to monitor the consequences of the treatment at multiple time points. Thus, standardized techniques to evaluate the effects of RA treatment in a quantitative, non-invasive, and longitudinal manner are critically needed.

Positron emission tomography (PET) with the radiotracer ^{18}F-fluorodeoxyglucose (^{18}F-FDG), a glucose analog, has been employed to quantify RA disease activity in small-animal models, including the CIA mouse model.[13] Furthermore, ^{18}F-FDG PET has been shown to be sensitive to changes in inflammatory activity due to RA drugs earlier than physical manifestations.[14] However, in these previous studies, the typical interval used to monitor therapeutic response was on the order of weeks after initiation of treatment. Our hypothesis was that ^{18}F-FDG PET will detect changes in inflammatory activity occurring in response to an RA drug during its peak and trough plasma levels, that is within mere hours of treatment. Our rationale was that such rapid assessment would contribute toward optimizing dosing regimens and evaluating time points for current and future RA treatments and early determination of treatment strategies that would be effective.

2 | METHODS

2.1 | Animals, group assignment and clinical scoring

All animal procedures were approved by our Institutional Animal Care and Utilization Committee, in accordance with the US Department of Agriculture animal care guidelines. CIA was induced in 24 male DBA/1 mice based on established protocols.[9] At day 42 after

the initial induction of CIA, the severity of the arthritis for each mouse paw was scored using the following algorithm: 0 = no erythema/swelling, 1 = 1 toe inflamed or swollen, 2 = more than 1 toe but not entire paw inflamed/swollen or mild swelling of the entire paw, 3 = entire paw inflamed or ankylosed paw. The clinical score of each mouse was taken as the summation of the scores for all four of its paws. Thus, the score ranged from 0 to 12 for each mouse.

Mice that were determined to have developed CIA (clinical score > 6) were selected and randomly assigned to 3 groups. Group 1 consisted of 13 mice (clinical score [mean ± SD] $9.00 ± 0.82$, at day 42). These animals were then treated with 1 dose of a JAK inhibitor tofacitinib,[15,16] 50 mg/kg, via oral gavage (PO).[17] These mice then underwent PET scanning (procedure below) at two time points (4 hours apart) after the treatment corresponding to the peak (60 minutes post-drug administration) and trough (4.5 hours post-drug administration) plasma levels of tofacitinib. These time points were based on Dowty et al[17] who showed in DBA/1 mice (same as used in this study) with a single PO dose of 50 mg/kg tofacitinib that the plasma concentrations for the drug peaked at around 1 hour after oral drug administration, and was a factor of over 6 lower at 4.5 hours after drug administration. The experimental timeline for this group is outlined in Figure 1. Group 2 consisted of 8 mice (clinical score $8.00 ± 2.16$, at day 42). These mice underwent PET scanning at 2 time points, before receiving tofacitinib treatment at day 42 (therefore tofacitinib-naïve), and after 6 days of receiving a single daily dosage of tofacitinib (50 mg/kg). Group 3 (control group) consisted of 3 mice (clinical score $9.00 ± 0.63$, at day 42). These mice were left untreated for the 6 days and received PET scans at 2 times to coincide with those received by Group 2.

2.2 | PET scanning

Mice received an intravenous injection of the ^{18}F-FDG radiotracer (~9.25 MBq) via the tail vein. The animals were then anesthetized with 2%−3% isoflurane and placed on a scanning bed of a small-animal PET scanner (Inveon DPET, Siemens Healthcare, Knoxville, TN, USA). PET scanning began 30 minutes after the ^{18}F-FDG injection, and lasted for 15 minutes. The vendor-provided ordered-subset-expectation-maximization method was used to reconstruct the PET images.

For Group 1, at 30 minutes after the administration of tofacitinib, the ^{18}F-FDG radiotracer was injected. Thirty minutes later (60 minutes after tofacitinib administration), the PET scan was performed, coinciding with the peak plasma level of tofacitinib. A second injection of ^{18}F-FDG was administered at 240 minutes from the time of tofacitinib administration, so that a 15-minute PET scan could be performed starting at 270 minutes, coinciding with the trough plasma level of tofacitinib. Given the radioactive half-life of [18]F-(109.7 minutes), not all radiotracer activity from the first injection would have decayed to background levels by 240 minutes. In order to compensate for this residual activity from the first injection, a PET scan was performed at 220 minutes, and PET measures thus derived were decay-corrected and subtracted from those from the 270 minutes scan.

For mice in Group 2, 2 PET scans were conducted using the aforementioned protocol, 1 before the start of treatment (day 42) and the 2nd after 6 days of administering the single

daily dose of treatment. Group 3 mice received no treatment and underwent PET scans to coincide with those from Group 2 (day 42 and 6 days later).

2.3 | Image analysis

PET images were analyzed using the PMOD tool (version 3.8, PMOD Technologies, Zurich, Switzerland). For the PET scan of each mouse, four volumes–of–interest (VOIs) were manually traced, encapsulating the hind right paw (HR), the hind left paw (HL), the fore right paw (FR) and the fore left paw (FL). The sizes of the VOIs remained unchanged throughout the study. For each limb VOI, the maximum standardized uptake value (SUV_{max}) was extracted, based on normalization by injected activity and animal weight. Two measures were calculated from each PET scan based on the SUV_{max} for the four paws:

1. maximum SUV_{max} ("Max SUV_{max}"): the highest SUV_{max} across the 4 paws, as a marker of disease severity, accounting for inflammatory activity of the most severely affected joint, and

2. average SUV_{max} ("Avg SUV_{max}"): sum of the SUV_{max} of each paw, divided by 4, as a quantitative measurement of the total inflammatory burden, analogous to the clinical score.

2.4 | Statistical analysis

Statistical analysis was performed using the R programming language (R Corporation, Vienna, Austria). The Max SUV_{max} and Avg SUV_{max} variables were compared using the Wilcoxon rank sum test, unpaired or paired, as appropriate. Statistical significance was assigned based on $P < .05$.

3 | RESULTS

3.1 | PET evaluates effect of the 6-day tofacitinib treatment

Group 3 mice that did not receive tofacitinib treatment showed insignificant change when comparing Max SUV_{max} before start of treatment (day 42: median = 1.51, range = [0.88–1.60] vs day 48: median = 1.36, range = [0.97–1.36]), or Avg SUV_{max} (day 42: median = 1.05, range = [0.77–1.06] vs day 48: median = 1.04, range = [0.81–1.09]). The clinical score also did not significantly change between the 2 points. These outcomes were consistent with previous studies showing insignificant changes in clinical and histological measures over this time frame.[14] The Max SUV_{max} in Group 2 mice before treatment (day 42) was 2.10 ± 0.75 (mean ± SD). The Avg SUV_{max} was 1.80 ± 0.61. The PET measures in groups 2 and 3 (both day 42) were significantly higher than animals of the same strain without CIA (Max SUV_{max} = 1.02 ± 0.05[13]).

At day 48 (after 6 days of tofacitinib treatment), there was a significant reduction in both the Max SUV_{max} and Avg SUV_{max} measures in Group 2 mice, in comparison to day 42. These results are summarized in Figure 2.

3.2 | PET assesses inflammatory activity at peak and trough plasma levels of tofacitinib

Our results are summarized in Figure 2, while representative PET images are shown in Figure 3. Both the Max SUV_{max} and Avg SUV_{max} measures showed a similar trend, undergoing a significant increase at trough tofacitinib plasma level (270 minutes) compared to peak tofacitinib plasma level (60 minutes) measures. Both PET measures at the 60 minutes time point were significantly lower than those measured for Group 2 pretreatment (day 42) and not significantly different from those obtained at day 48 (after 6 days of daily tofacitinib treatment). On the other hand, the PET measures from the 270 minutes time point were significantly higher than those obtained for Group 2 mice at day 48 (6 days of daily tofacitinib treatment) but were not significantly lower than those from Group 2, at day 42.

The Avg SUV_{max} showed a similar trend as the Max SUV_{max} over all 4 time points, showing increase in Avg SUV_{max} as the level of tofacitinib went from peak value (60 minutes) to trough value (270 minutes). The median Avg SUV_{max} was significantly lower for the 60 minutes time point in comparison to pretreatment (Group 2, day 42).

4 | DISCUSSION AND CONCLUSIONS

The aim of this study was to assess the effectiveness of ^{18}F-FDG-PET to quantify inflammation activity in CIA mice treated with tofacitinib, a JAK inhibitor. Based on our results, we accept the hypothesis that ^{18}F-FDG PET can detect changes in inflammatory activity occurring in response to an RA drug during its peak and trough plasma levels, that is within mere hours of treatment to several days of treatment. To our knowledge, this is the first study to use ^{18}F-FDG-PET to monitor inflammation levels within the first few hours after administration of RA treatment.

The significant increase in PET measures from the 60 to 270 minutes time points suggests that greater inflammatory activity occurs when the plasma levels of tofacitinib have decreased to trough levels. It is expected that decreased tofacitinib level will coincide with an increase in inflammation; however, this study is unique because it showed that ^{18}F-FDG-PET can be used to quantify and correlate inflammation levels with the peak and trough plasma levels of an effective anti-inflammatory drug. After 6 days of daily tofacitinib exposure, our data show that PET measures are significantly reduced compared to baseline, indicating that inflammatory activity can eventually be arrested. These results support the demonstration that adaptation of both the signaling and transcriptional networks occurs with repeated JAK blockade compared to a single administration, with persisting drug effect after the drug's clearance.[18] Furthermore, these results show that rapid screening and further optimization of dosing regimens and evaluation time points may be enabled by ^{18}F-FDG-PET.

Our study had a few limitations. First, our PET evaluation time frame for Group 2 was on days 42 and 48, that is after exposure to a daily dose of tofacitinib for 6 days. We are therefore not able to predict the exact kinetics of inflammatory activity between these time points (for example, at day 45), which will be a subject of further study. This could help generate evidence-based data for implementing in clinical studies. Second, it remains unknown if the reduction in inflammatory activity after JAK inhibition is sustained after 6

days, or if continued treatment is necessary, and at what dosage level. This information could be useful for assessing dosing regimens in RA patients and addressing co-morbidities and adverse events.

Overall, our results suggest that ^{18}F-FDG PET provides a robust means to rapidly and quantifiably track response to tofacitinib at time scales of a few hours to several days. This study has implications for future RA drug research, as it provides a template to rapidly test candidate treatments. In an analogous manner, ^{18}F-FDG PET would enable studies of the same drug in human RA patients and motivate co-clinical trials. Finally, there would be opportunities to evaluate if objective assessments via PET could overcome challenges of heterogeneity of response commonly present in RA patients.[19]

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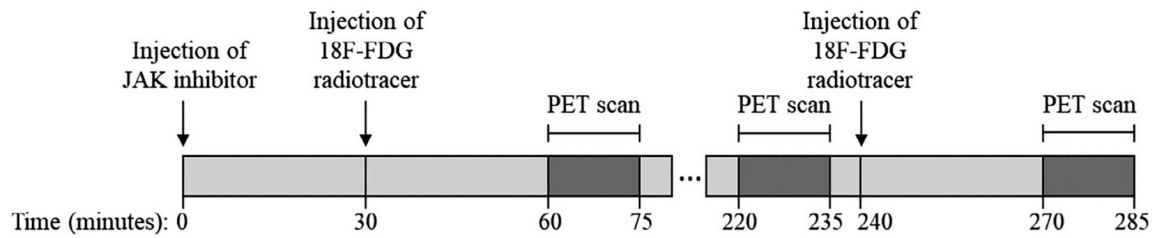
FIGURE 1.
Experimental timeline for the collagen-induced arthritis (CIA) mice in Group 1. JAK, Janus-activated kinase; ^{18}F-FDG, ^{18}F-fluorodeoxyglucose; PET, positron emission tomography

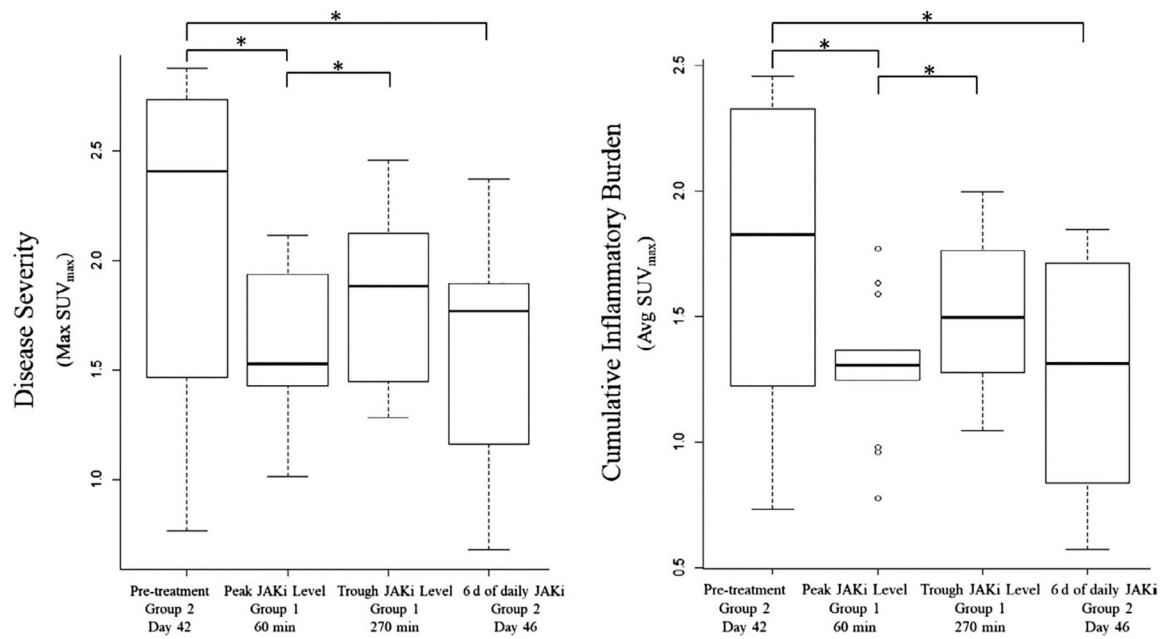**FIGURE 2.**
Comparison of extremity maximum standardized uptake value maximum (Max SUV_max) and average standardized uptake value (Avg SUV_max) scores at peak versus trough Janus-activated kinase inhibitor (JAKi) levels (Group 1), and before and after 6 days of treatment (Group 2)
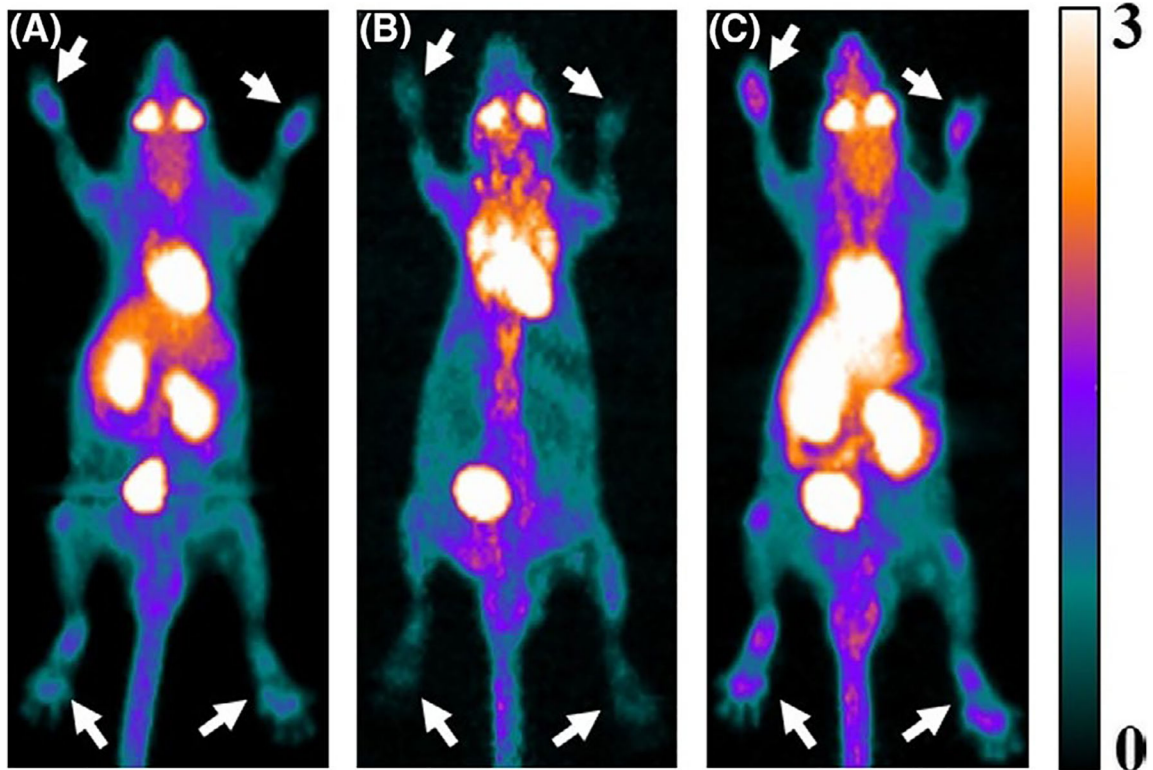
FIGURE 3.
Representative positron emission tomography (PET) maximum intensity projection images of a mouse from Group1 at (A) 60 minutes, (B) 220 minutes, and (C) 270 minutes post-tofacitinib administration. The color bar indicates the standardized uptake values (SUV) scale. White arrows indicate the regions evaluated