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Targeted PET Imaging of Chemokine Receptor 2+ Monocytes and Macrophages in the Injured Heart

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Short title: PET imaging CCR2 in injured heart

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Proinflammatory macrophages are important mediators of inflammation following myocardial infarction and allograft injury following heart transplantation. The aim of this study was to image the recruitment of proinflammatory chemokine receptor 2+ (CCR2+) cells in multiple heart injury models. **Methods:** ^{64}Cu-DOTA-ECL1i PET was used to image CCR2+ monocytes/macrophages in heart transplantation mouse model. Flow cytometry was performed to characterize CCR2+ cells. Autoradiography on human heart specimen was conducted to confirm binding specificity. ^{64}Cu-/^{68}Ga-DOTA-ECL1i were compared in ischemia/reperfusion injury mouse model. **Results:** ^{64}Cu-DOTA-ECL1i showed sensitive and specific detection of CCR2+ cells in all tested mouse models with comparable efficacy to ^{68}Ga-DOTA-ECL1i. Flow cytometry demonstrated specific expression of CCR2 on monocytes/macrophages. The tracer binds to human CCR2. **Conclusion:** This work establishes the utility of ^{64}Cu-DOTA-ECL1i to image CCR2+ monocytes/macrophages in mouse models and provides the requisite pre-clinical information to translate the targeted clinical grade CCR2 imaging probe for clinical investigation of heart diseases.

Key Words: PET, C-C Chemokine Receptor type 2 (CCR2), monocytes, macrophages, heart injury

INTRODUCTION

Within the heart, macrophages represent the most abundant immune cell type and have important roles in potentiating both inflammatory and reparative responses following cardiac injury. Recent studies have shown that chemokine receptor 2+ (CCR2+) monocytes and macrophages, recruited to the site of myocardial injury, are important mediators of adverse remodeling following myocardial infarction (MI), heart failure pathogenesis, and inflammation after heart transplantation. Thus, CCR2+ cells represent a potential therapeutic target to improve outcomes in the above disease settings (*1-3*).

Though some PET tracers have been developed for MI imaging (*4-6*), these probes lack specificity visualizing cell types important to the remodeling process. Molecular imaging of CCR2+ monocytes and macrophages provides not only a sensitive approach to determine a specific mechanism of myocardial inflammation that is therapeutically targetable, but also a strategy to facilitate the selective delivery of immunomodulatory agents to cardiovascular patients that are most likely to benefit.

Previously, we reported the capacity of a targeted PET tracer to quantify the CCR2-specific inflammation burden in multiple animal models using ECL1i peptide through ^{64}Cu or ^{68}Ga radiolabel (*7-9*). The PET signal intensity in inflammatory tissues was corroborated by molecular characterization of CCR2 expression and correlated with disease progression and regression. Currently, ^{64}Cu radiolabeled ECL1i (^{64}Cu-DOTA-ECL1i) is approved by FDA for clinical investigations to identify CCR2+ cells in humans (*10*). To explore its potential imaging patients with heart injury, we assessed the sensitivity and specificity of ^{64}Cu-DOTA-ECL1i CCR2+ monocyte and macrophage abundance in pre-clinical models of heart transplantation and MI, and compared to ^{68}Ga radiolabeled analog. These studies provided strong support to apply CCR2 PET imaging strategy in human subjects.

MATERIALS AND METHODS

Mouse Heart Injury Models

For the heart transplantation mouse model, cardiac grafts harvested from wild type (WT) C57BL/6 mice were transplanted into the right neck of C57BL/6 LysM-GFP or CCR2 knockout ($CCR2^{-/-}$) recipient mice following 1 h of cold (4 °C) ischemia as previously described (*11*). The ischemia/reperfusion injury mouse model was developed in 2-4-month-old C57BL/6 mice following a reported protocol (*9*).

Radiolabeling of DOTA-ECL1i with ^{64}Cu and ^{68}Ga

The synthesis of DOTA-ECL1i and radiolabeling with ^{64}Cu and ^{68}Ga were reported previously (*9,12*). The radiochemical purities for both tracers were more than 95% prior to the administration to mice.

PET/CT Imaging and Biodistribution

All animal studies were performed in compliance with guidelines set forth by the NIH Office of Laboratory Animal Welfare and approved by the Washington University institutional animal care and use committee. At 1-2 h post heart transplantation (*2,13*), PET/CT (Siemens, Malvern, PA, 40 to 60 min dynamic scan) with ^{64}Cu-DOTA-ECL1i was performed following tail vein injection. For mice with ischemia/reperfusion injury, PET/CT scan was done at day 3 post injury with ^{68}Ga-DOTA-ECL1i and at day 4 with ^{64}Cu-DOTA-ECL1i and quantified as reported (*9,12*). Post-PET biodistribution study in the heart transplant model was performed immediately after imaging (*8*).

Flow Cytometry

To characterize the expression of CCR2, saline perfused cardiac tissue specimens were collected and processed as previously reported. FACS analysis was performed on BD LSR II platform (*9*).

Autoradiography

Human pathologic specimens were obtained under Institutional Review Board protocol (#201305086) approved by Washington University Human Research Protection Office. Fixed human heart tissue sections were used to test the binding of ^{64}Cu-DOTA-ECL1i to human CCR2. Competitive receptor blocking studies were also performed to verify the binding specificity following previous reports (*8,9*).

Statistics

Data were analyzed using GraphPad Prism (version 6.07, La Jolla, California). Differences between groups were compared by using paired parametric or unpaired nonparametric two-tailed t tests. $P < 0.05$ was indicative of a statistically significant difference.

RESULTS

The sensitivity and specificity of ^{64}Cu-DOTA-ECL1i to image CCR2+ cells were assessed in mouse syngeneic heterotopic heart transplant models. In this model, hearts from wild type mice were harvested, placed on ice for 1 hour, and subsequently transplanted in the cervical position of either a wild type recipient mouse or CCR2$^{-/-}$ mouse. As shown in Figure 1A, besides rapid renal clearance as previous reported (*12*), PET/CT imaging revealed intensive signal in the donor heart but minimal tracer retention in the native heart in the WT→WT mouse heart transplantation model. In the WT→CCR2$^{-/-}$ model (Figure 1B), a similar profile was detected with tracer uptake in the donor heart and marginal accumulation in the native heart. Quantitative uptake analysis showed that tracer accumulation in the donor heart (3.51±0.19%ID/gram, n=4) was approximately 4 times higher than that determined in the native heart (0.72±0.08%ID/gram, $p<0.0001$, n=4) in the WT→WT model (Figure 1C). In the WT→CCR2$^{-/-}$ model (Figure 1C), 5-fold higher tracer accumulation difference was determined in donor hearts (1.46±0.16%ID/gram, n=3) than that acquired in native hearts (0.25±0.06%ID/gram, $p<0.0001$, n=3). Moreover, the donor heart tracer uptake in the WT→WT model was 1.4-fold ($p<0.0001$, n=3-4) higher than that in the WT→CCR2$^{-/-}$ model while the difference was approximately 5-fold ($p<0.0001$, n=3-4) for the native hearts in the two models, which was likely a result of reduced infiltration of recipient CCR2+ cells from CCR2$^{-/-}$ recipients into the graft as was reported in the ischemia/reperfusion injury model after lung transplantation (*12*). Of note, tracer uptake was evident within donor hearts transplanted into CCR2$^{-/-}$ recipients. This is likely due to the persistence of CCR2+ resident macrophages following transplantation, a population of cells that are activated following ischemia reperfusion injury and orchestrate infiltration of peripheral monocytes and neutrophils (*2,13*).

We have previously demonstrated that recipient CCR2+ monocytes and macrophages accumulated within the transplanted heart following transplantation (*2*). In agreement, flow cytometry analysis of the WT→WT model revealed abundant CCR2+ monocytes and macrophages in the transplanted donor heart as compared to the native heart 2 days following heterotopic heart transplantation (Figure 1D). Furthermore, post PET biodistribution showed that tracer uptake in the donor heart (2.82±0.40%ID/gram) was 4 times as much as that in the native heart (0.70±0.19%ID/gram, $p<0.0001$, n=4) (Figure 1E), supporting PET data. In contrast to the bone marrow uptake (0.57±0.16%ID/gram, n=4) in naïve mice (*8*), tracer localization in the marrow (2.33±0.51%ID/gram) of WT→WT recipient mouse was also 3 times higher, indicating the increased production and accumulation of CCR2+ monocytes within the bone marrow compartment.

To further assess the potential of ^{64}Cu-DOTA-ECL1i for human cardiac injury imaging, the binding of ^{64}Cu-DOTA-ECL1i to human CCR2 was evaluated in human MI specimens, which are known to contain CCR2+ monocytes and macrophages, using autoradiography (*9*). A substantial and heterogeneous binding profile was observed in human MI tissues, which was blocked by excess non-radioactive ECL1i peptide (Figure 1F), indicating the specificity of ^{64}Cu-DOTA-ECL1i binding to human hearts.

To further assess the suitability of ^{64}Cu-DOTA-ECL1i for human cardiac injury imaging, we compared it to ^{68}Ga radiolabeled analog in a previously established mouse MI model (*9*). ^{68}Ga-DOTA-ECL1i and ^{64}Cu-DOTA-ECL1i each showed significant radiotracer uptake within the infarct region 3-4 days following ischemia/reperfusion injury (Figure 2A, B). Quantitative analysis in the same mouse showed that the uptake of ^{68}Ga-DOTA-ECL1i within the heart at day 3 post-injury (3.07 ±0.33%ID/gram, n=6) was comparable to that acquired a day later using ^{64}Cu-DOTA-ECL1i (2.71±0.28%ID/gram, n=6). These data are also comparable with ^{68}Ga-DOTA-ECL1i uptake at day 4 post MI (2.53±0.52%ID/g, n=6) (*9*), indicating equivalent sensitivity of ^{64}Cu-DOTA-ECL1i and ^{68}Ga-DOTA-ECL1i for detecting CCR2+ cells within the infarcted heart.

DISCUSSION

Clinically, the identification of reliable biomarkers of inflammation is required to facilitate a timely diagnosis of heart transplant rejection and primary graft dysfunction, identify heart failure patients at increased risk of disease progression, elucidate pathways that contribute to the pathogenesis of these entities, and allow for the development and appropriate utilization of immunomodulatory therapies. It is known that CCR2+ macrophages generate damaging inflammation by recruiting additional monocytes and neutrophils that release cytokines and oxidative products. Removal of CCR2+ macrophages is sufficient to reduce chemokine and cytokine production, monocyte and neutrophil recruitment, and results in preservation of left ventricular function and suppression of pathological remodeling following MI and heart transplantation. Thus, the precise imaging of CCR2+ macrophages has the potential to determine the severity of heart injury, provide guidance for targeted treatment, and monitor treatment response.

Herein, we report robust detection of CCR2+ monocytes and macrophages in mouse models of heart injury using a clinical grade ^{64}Cu-DOTA-ECL1i PET tracer. The imaging sensitivity and specificity were demonstrated using WT→WT and WT→CCR2$^{-/-}$ heart transplantation models. We demonstrated that the ^{64}Cu-DOTA-ECL1i tracer provides equivalent imaging efficacy compared to our previously published ^{68}Ga-DOTA-ECL1i tracer in a mouse MI model. We further demonstrated that the ^{64}Cu-DOTA-ECL1i tracer binds inflamed human myocardial tissue.

Compared to ^{68}Ga, the radioactive decay half-life of ^{64}Cu is 10 times longer, providing an imaging agent that is ideal for multicenter translational studies. ^{64}Cu-DOTA-ECL1i can be produced at a single center and intact tracer shipped to collaborating institutions, avoiding the need for onsite ^{68}Ge/^{68}Ga generators or radiochemistry expertise necessary for local tracer production. More importantly, the ^{64}Cu-DOTA-ECL1i tracer is currently approved by US FDA for clinical investigation under an exploratory investigational new drug application.

CONCLUSION

In conclusion, this study demonstrates the utility of ^{64}Cu-DOTA-ECL1i to detect CCR2+ monocytes and macrophages in pre-clinical models and provides the requisite pre-clinical data to support translation of ^{64}Cu-DOTA-ECL1i for clinical investigation of heart injuries.

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DISCLUSURES

Y. Liu, D. Kreisel, R.J. Gropler, S.L. Brody, and K.J. Lavine have a pending patent entitled "Compositions and Methods for Detecting CCR2 Receptors" (application No. 15/611,577). D. Kreisel serves on the Scientific Advisory Board of Compass Therapeutics. The other authors report no conflicts.

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KEY POINTS

QUESTION: What is the feasibility to non-invasively image proinflammatory chemokine receptor 2+ cells after heart injury?

PERTINENT FINDINGS: ^{64}Cu-DOTA-ECL1i showed sensitive and specific detection of CCR2+ cells in mouse heart injury models, which was further confirmed by flow cytometry analysis. The tracer also binds to injured human heart specimens.

IMPLICATIONS FOR PATIENT CARE: This study provides the requisite pre-clinical data to support translation of ^{64}Cu-DOTA-ECL1i for clinical investigation of heart injuries and may provide new insight into the role of inflammation during human heart failure progression.

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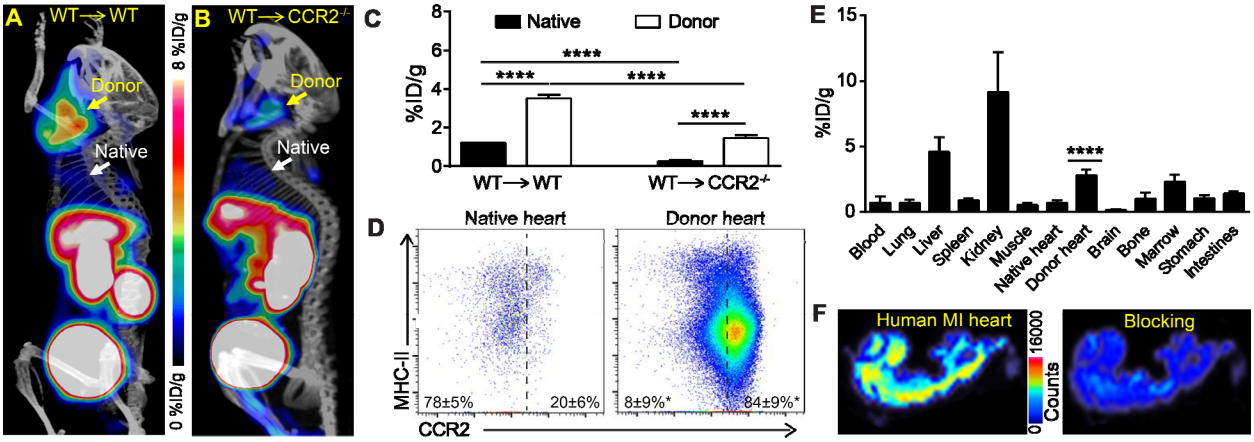

FIGURE 1. ^{64}Cu-DOTA-ECL1i imaging CCR2 in heart transplantation models. ^{64}Cu-DOTA-ECL1i PET/CT images (maximum intensity projection) showed significant signal in the transplanted heart (yellow arrow) and weak accumulation in the native heart (white arrow) in (A) WT→WT model and (B) WT→CCR2$^{-/-}$ model. (C) Quantitative uptake analysis of native and donor hearts of the two models (WT→WT and WT→CCR2$^{-/-}$). (D) Flow cytometry assay showed three-fold more CCR2+ cells in the transplanted heart than those determined in the native heart in the WT→WT model. (E) Post-PET biodistribution showed 3-fold higher uptake in donor heart compared to native heart in the WT→WT model. (F) Autoradiographic images of ^{64}Cu-DOTA-ECL1i on human MI specimens revealed heterogeneous tracer binding. Competitive blocking using excess nonradioactive ECL1i showing decreased tracer binding. **** $p<0.0001$, assessed by one-way ANOVA.

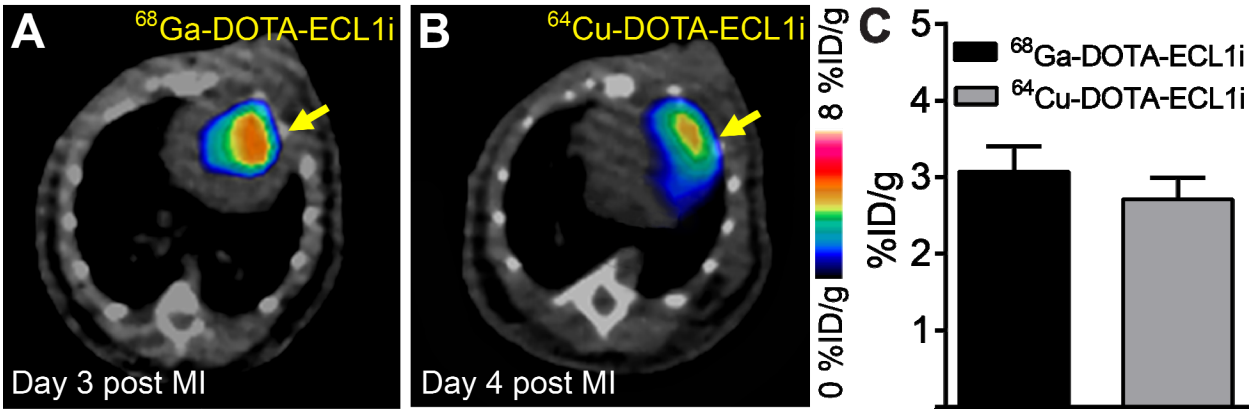

FIGURE 2. ^{64}Cu-DOTA-ECL1i compared to ^{68}Ga-DOTA-ECL1i-mediated imaging CCR2 in the injured heart. Representative PET/CT images (transverse plane) of ^{68}Ga-DOTA-ECL1i acquired at day 3 (A) and ^{64}Cu-DOTA-ECL1i acquired at day 4 (B) post MI in the same mouse showing comparable PET signals (**C**) in the same infarcted region of heart.