[^{68}Ga]Ga-THP-Pam: A PET radiotracer for imaging vascular calcification

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Introduction

[^{68}Ga]Ga-THP-Pam was previously demonstrated to have high affinity towards a number of calcium salts while [^{18}F]NaF, the most used PET radiotracer for bone imaging has high affinity only for hydroxyapatite (the main component of bone mineral).[1] It was hypothesised that the broad calcium mineral affinity of [^{68}Ga]Ga-THP-Pam may be advantageous in detection of vascular calcification (VC), where the composition of solid calcium mineral may be more varied than the composition of bone.[2] A direct comparison of [^{68}Ga]Ga-THP-Pam and [^{18}F]NaF in a rat model of VC was performed to test this hypothesis.

Methods

A model of VC was used in which rats were fed a diet containing warfarin and vitamin K_1 along with subcutaneous administration of vitamin D_3 to induce severe VC.[3] Anaesthetised rats were injected with [^{68}Ga]Ga-THP-Pam and scanned using preclinical PET/CT 60–120 min post-injection. The rats were imaged using the same protocol with [^{18}F]NaF the following day. As a control study, animals fed a healthy diet were imaged using the same procedure. Organs were harvested and their radioactivity was measured for *ex vivo* biodistribution. Organs of interest were fixed in formalin and embedded in paraffin. Paraffin-embedded organs were scanned using μCT.

Results

Imaging (Figure 1A) showed high uptake of [^{68}Ga]Ga-THP-Pam and [^{18}F]NaF (3.44 ± 0.69 and 0.91 ± 0.24 %ID respectively, p = 0.002, Figure 1B) in a region of tissue around the stomach, with severe calcification as identified by CT. Additionally, [^{68}Ga]Ga-THP-Pam demonstrated increased uptake in the VC group *vs.* the healthy group across several major organs, most notably in the kidneys (2.21 ± 0.76 *vs.* 0.25 ± 0.13 %ID, p = 0.002, Figure 1B). *Ex vivo* biodistribution data confirmed the increased uptake of [^{68}Ga]Ga-THP-Pam observed in the imaging data. The presence of calcification in the kidneys, stomach and other organs of interest was confirmed by microCT-based 3D X-ray histology (XRH) and conventional histology (Figure 1C). To visualise small areas of interest such as the aorta, a prototype post-reconstruction method to improve the spatial resolution of preclinical PET with gallium-68 was used. Analysis of the mineral composition of the calcifications is ongoing.

Conclusions

These results demonstrate that [^{68}Ga]Ga-THP-Pam may offer improved detection of VC in comparison to [^{18}F]NaF, including microcalcifications undetectable by preclinical CT.

References

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2. E. Tsolaki and S. Bertazzo, *Materials*, 2019, **12**, 3126.
3. J. Bordoloi, PhD Thesis, King's College London, 2015.

Figure

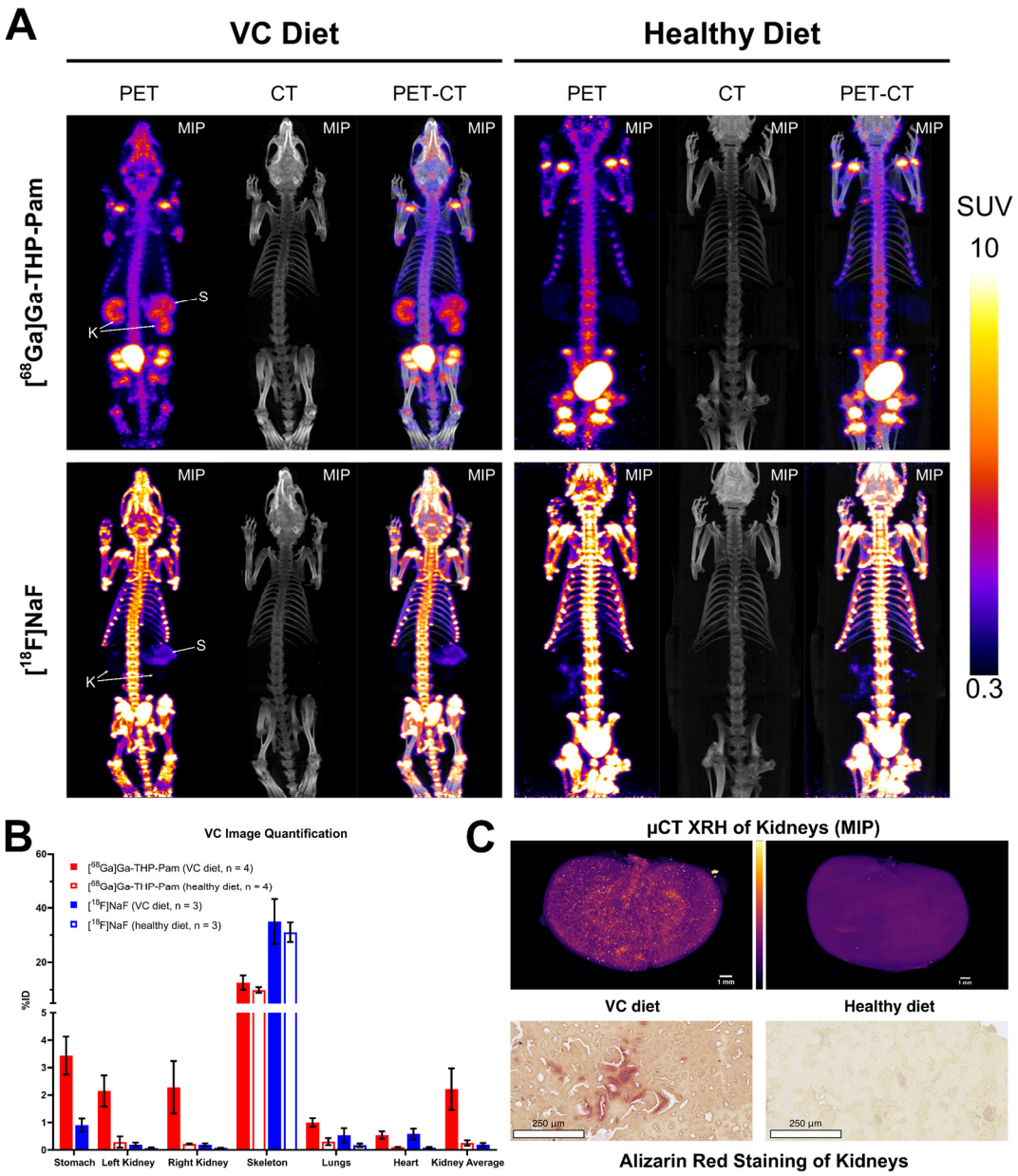
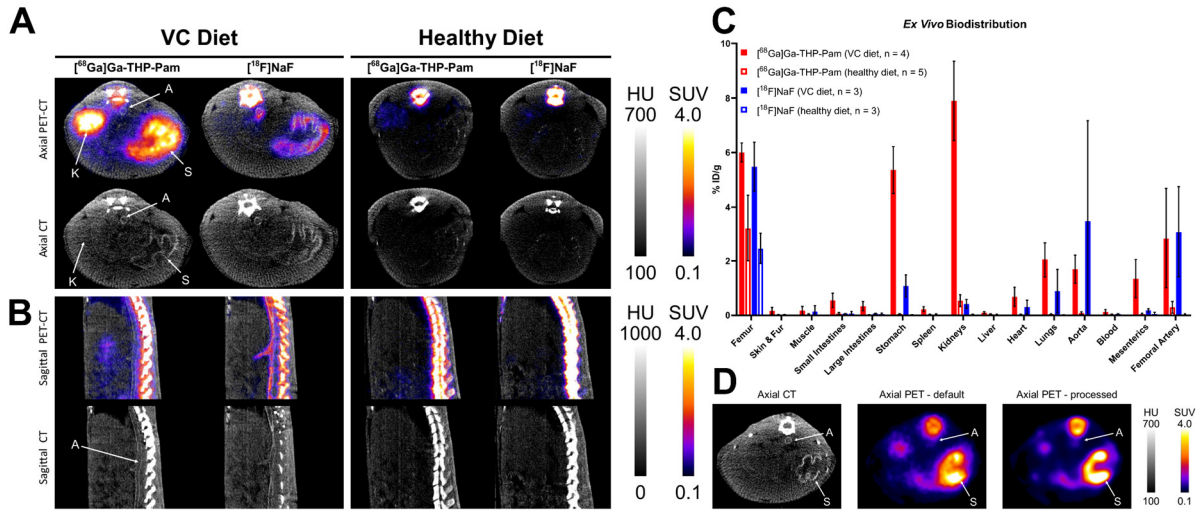


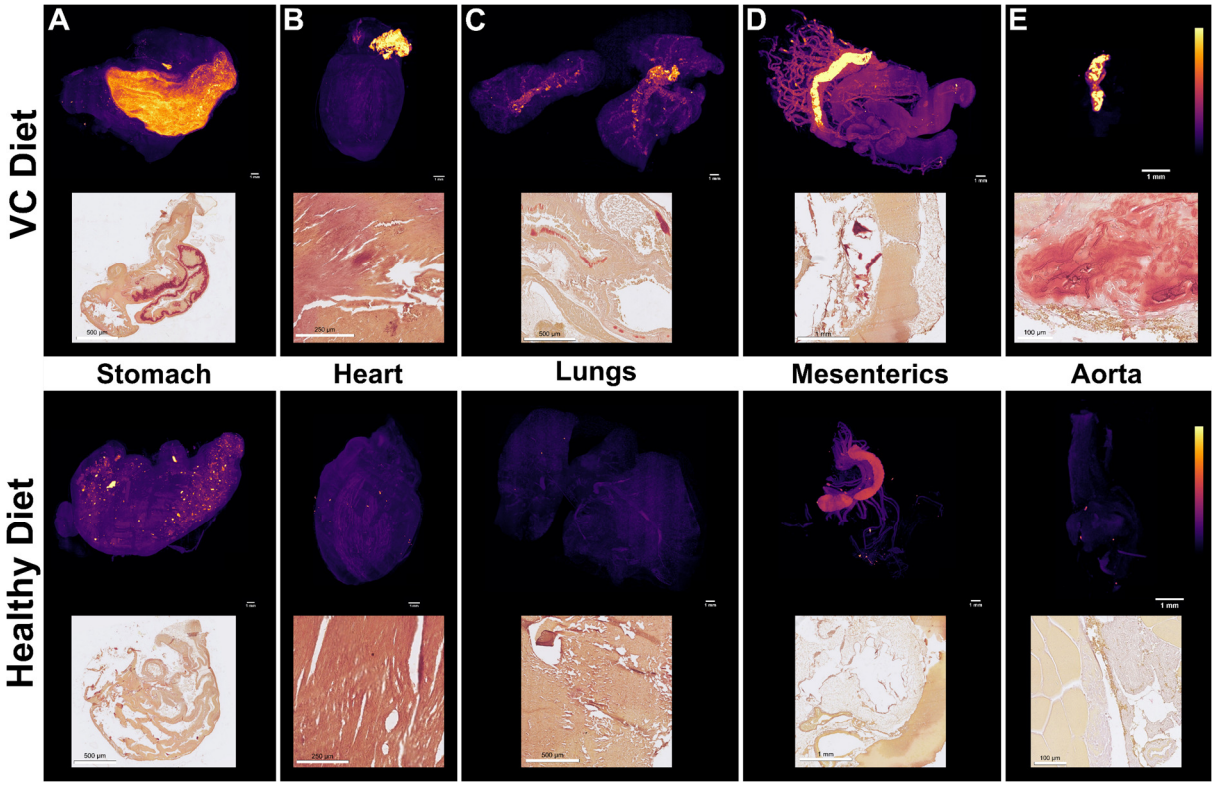
Figure 1. (A) PET-CT MIP images of rats with vascular calcification and control rats with both [^{68}Ga]Ga-THP-Pam and [^{18}F]NaF 60–120 min post-injection. The images of calcified rats are both of the same rat, and the control images are also data from the same rat. Each PET image shows a standard uptake value (SUV) scale of 0.3–10. S = stomach. K = kidneys. (B) Quantification from PET images of [^{68}Ga]Ga-THP-Pam and [^{18}F]NaF in %ID in rats fed a diet to induce VC and rats fed a healthy diet. (C) E*x vivo* detection of calcification in kidneys

by μCT XRH (top panel, colour scales matched) and conventional histology with Alizarin Red staining (bottom panel) in kidneys from rats from the VC group and the healthy group.

Persuasive Data file



Additional biodistribution data. (A&B) PET-CT and corresponding CT slices of regions of interest in rats from the VC group and the healthy group 60–120 min post-injection of [^{68}Ga]Ga-THP-Pam and [^{18}F]NaF. (A) Axial slices through the stomach region, highlighting the visibility of the stomach/tissue and aorta on CT in the VC group, but no calcification is visible in the kidney by CT. (B) Sagittal slices showing the abdominal aorta. Empty space around all images has been cropped to fit the panel and size may not be directly comparable. (C) *Ex vivo* biodistribution 2 hours post-injection of [^{68}Ga]Ga-THP-Pam and [^{18}F]NaF in %ID/g in rats fed a diet to induce VC and rats fed a healthy diet. (D) Axial slices from a PET-CT scan showing the CT, PET image ([^{68}Ga]Ga-THP-Pam) using only the default reconstruction method and processed PET image. S = stomach; A = aorta; K = kidneys.



µCT confirms calcification in VC diet group. µCT XRH MIPs of paraffin-embedded organs of interest *ex vivo* and corresponding histological staining with Alizarin Red, top panel shows VC diet, bottom panel shows healthy diet. Each organ comparison is shown with matched colour scales. (A) Stomach. (B) Heart. (C) Lungs. (D) Mesenterics. (E) Aorta.