PET/MR imaging of hypoxic atherosclerosis using $^{64}$Cu-ATSM in a rabbit model

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Short running title

$^{64}\text{Cu-ATSM}$ imaging of hypoxic plaque
ABSTRACT

The macrophage-rich core of advanced human atheroma has been demonstrated to be hypoxic, which may have implications in plaque stability. The goal of this study was to determine the feasibility of the hypoxia positron emission tomography (PET) imaging agent $^{64}$Cu-ATSM to detect hypoxia in a rabbit model of atherosclerosis imaged on a simultaneous positron emission tomography/magnetic resonance (PET/MR) scanner, using MR for both attenuation correction and depiction of lesion location.

Methods

New Zealand White rabbits fed a Western diet for 4-6 wk underwent endothelial denudation of the right femoral artery by air desiccation to induce an atherosclerotic-like lesion, and underwent a sham operation on the left femoral artery. Four and 8 wk after injury, a 0-60 min dynamic whole-body PET/MR exam was performed post-injection of ~111 MBq $^{64}$Cu-ATSM. After 24 h, a 0-75 min dynamic PET/MR exam post-injection of ~111 MBq $^{18}$F-FDG was performed. The rabbits were euthanized and the injured femoral artery (IF) and sham-operated femoral artery (SF) were collected for immunohistochemistry assessment of hypoxic macrophages (hypoxia marker pimonidazole, macrophage marker RAM-11, and hypoxia-inducible factor-1 alpha subunit HIF-1α). Regions of interests of IF, SF and background muscle (BM) were drawn on fused PET/MR images and
IF/BM and SF/BM standardized uptake values ratios were compared using Student’s t test.

Results

Elevated uptake of $^{64}$Cu-ATSM was found in the rabbits’ IF compared to the SF. $^{64}$Cu-ATSM imaging demonstrated IF/SF mean standardized uptake value (SUV$_{\text{mean}}$) ratios ($\pm$ standard deviation) of $1.75 \pm 0.21$ and $2.30 \pm 0.26$ 4 and 8 wk after injury, respectively. $^{18}$F-FDG imaging demonstrated IF/SF SUV$_{\text{mean}}$ ratios of $1.84 \pm 0.12$ 8 wk after injury. IF/BM SUV$_{\text{mean}}$ ratios were significantly higher ($P < 0.001$) than SF/BM SUV$_{\text{mean}}$ ratios both 4 and 8 wk after injury for $^{64}$Cu-ATSM and 8 wk after injury for $^{18}$F-FDG ($P < 0.05$). Pimonidazole immunohistochemistry at 8 wk co-localized to RAM-11 and HIF-1$\alpha$.

Conclusion

The results show that hypoxia is present in this rabbit model of atherosclerosis and suggest that $^{64}$Cu-ATSM PET/MR is a potentially promising method for the detection of hypoxic and potentially vulnerable atherosclerotic plaque in human subjects.

Keywords

$^{64}$Cu-ATSM, PET/MR, hypoxic atherosclerosis, rabbit model
INTRODUCTION

Atherosclerosis is a systemic degenerative and inflammatory vascular disease that develops over decades, leading to advanced lesions characterized by a lipid core separated from the lumen by a fibrous cap. It has been recognized that plaque composition more than the degree of luminal stenosis determines the risk of acute clinical events (e.g. stroke, myocardial infarction) (1). Macrophage-rich plaques with a thin fibrous cap, large lipid core, and abundance of leaky microvessels tend to be more vulnerable (1). The rupture or erosion of the fibrous cap in vulnerable plaque may lead to thromboembolization and arterial occlusion (2,3).

According to the anoxemia theory of atherosclerosis, an imbalance between the demand for and supply of oxygen in the arterial wall is a key factor in the development of atherosclerotic lesions (4). In the initial stages of atherogenesis, modified lipoproteins recruit monocytes and T cells. Macrophages internalize the modified lipoproteins resulting in the accumulation of high oxygen-consuming, high-metabolic-rate, lipid-loaded macrophages (foam cells) in developing lesions (5,6). The combination of increased oxygen demand together with impaired oxygen diffusion capacity results in the presence of severe hypoxia (<1% oxygen) in macrophage-rich zones (150-300 µm) into the lesion (7-9). There is growing evidence that zones of hypoxia occur at depth in the atherosclerotic plaque (6,8). Leppanen et al. (6) suggests that over time macrophages within the plaque core become ATP-depleted and severely hypoxic, contributing to their...
death and formation of a necrotic core that increases angiogenesis and plaque
destabilization. Sluimer et al. (9) demonstrates the direct presence of hypoxia in
the macrophage-rich regions of advanced human carotid atherosclerotic plaques
using the hypoxia marker pimonidazole, correlating hypoxia with CD68-positive
macrophages, and angiogenesis.

Our study investigated the hypoxia PET imaging agent $^{64}$Cu-ATSM in a
PET/MR hybrid scanner for detecting hypoxic atherosclerosis in an animal model.
The PET/MR imaging strategy uses MRI for attenuation correction and for co-
localization of $^{64}$Cu-ATSM uptake on PET to the rabbit femoral artery lesion
depicted anatomically on a 3 Tesla MR image. $^{64}$Cu-ATSM is of particular interest
because advanced atheromas in human carotids leading to angiogenesis and
thrombosis (i.e. unstable or vulnerable plaques) have been shown to be hypoxic
(9). Thus, this hypoxic cell-avid PET agent, which is already in use in human
patients for imaging tumor hypoxia, has the potential to translate into human
subjects for atherosclerosis imaging. In this paper, we show that $^{64}$Cu-ATSM can
detect hypoxia in an animal model with atherosclerotic-like lesions and that
hypoxia in these lesions as determined by $^{64}$Cu-ATSM uptake on PET and
pimonidazole staining of the ex-vivo specimen obtained after imaging co-localizes
to macrophages.
MATERIALS AND METHODS

General

Animal studies were performed under a protocol approved by the Animal Studies Committee at our institution. Advanced atherosclerotic-like lesions were induced in the right femoral arteries of 5 New Zealand White rabbits weighing 2.5-3 kg by endothelial denudation with air desiccation 2-4 wk after the start of a Western diet (0.25% cholesterol, Purina TestDiet, Indianapolis, TN) (10,11), depending on when the serum cholesterol level of the rabbits exceeded 2 mg/mL. The left femoral artery underwent a sham operation as the negative control. At the end of the last imaging procedure, the anesthetized animals were euthanized by exsanguination and the arterial segments collected for immunohistochemistry.

All chemicals, unless otherwise stated, were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO). Water was distilled and then deionized (18MΩ/cm²) by passing through a Milli-Q water filtration system (Millipore Corp., Milford, MA). ⁶⁴Cu was prepared as previously described (12). Radioactivity was counted with a Beckman Gamma 8000 counter containing a NaI crystal (Beckman Instruments, Inc., Irvine, CA). EM Science Silica Gel 60 F254 thin-layer chromatography (TLC) plates (10 × 5 cm) were purchased from EMD Millipore Corporation (Billerica, MA). Radioactive thin-layer chromatography plates were analyzed using a BIOSCAN System 200 plate reader (Bioscan, Inc., Washington, DC).
Diacetyl-bis (N⁴-methylthiosemicarbazonate) [H₂-ATSM] was labeled with ⁶⁴Cu as per literature methods (13). Briefly, 1 mg of ASTM powder was dissolved in 1 mL of dimethyl sulfoxide (DMSO) to give an overall concentration of 1 mg/mL. A total of 10 µL (10 µg) of this solution was then added to 4 µL (~370 MBq) of ⁶⁴CuCl₂ in 200 µL of 1 M NaOAc or 3 M HCl buffer. This solution was then stirred for 3 to 5 minutes and then allowed to sit briefly at room temperature, after which it was loaded onto a C₁₈ Sep-Pak® Light (Waters Corporation, Milford, MA), which had been pre-conditioned with 5 mL of ethanol and 5 mL of water. After loading the sample, 10 mL of water was passed through to remove the dimethyl sulfoxide and any unreacted ⁶⁴Cu. The labeled complex was then eluted in 350 µL of ethanol (following a 150 µL ethanol elution for the void volume), from which ~111 MBq (3 mCi) of the compound was extracted and diluted into 3 mL saline prior to injection. The purity of the labeled material was determined by radioactive thin-layer chromatography plates using silica gel plates with ethyl acetate as the mobile phase. Radiochemical yield was 80-85% and purity >95%.

**Imaging protocol**

The rabbits underwent simultaneous PET/MR (Siemens Biograph mMR) examination 4 and 8 wk after arterial injury operation (Fig. 1). At each time-point, a 0-60 min dynamic whole-body PET/MR exam was performed post-injection (p.i.) of ~111 MBq ⁶⁴Cu-ATSM. At the second time point, an additional 0-75 min dynamic PET/MR exam p.i. of ~111 MBq ¹⁸F-FDG was performed.
During PET acquisition, MR scans consisted of T₁-weighted 2D turbo-spin echo (TSE) (TR/TE = 600/11 ms, number of slices = 16, field of view = 71×120 mm, matrix size = 456×768), T₂-weighted 2D TSE (TR/TE = 2200-2890/56 ms, number of slices = 16, field of view = 60×120 mm, matrix size = 384×768), and proton density (PD)-weighted 2D TSE (TR/TE = 2200-2890/11 ms, number of slices = 16, field of view = 60×120 mm, matrix size = 384×768) were obtained. For all sequences, magnetic field strength = 3T, pixel bandwidth = 260 kHz, flip angle = 124°-151°, echo train length = 7, in-plane resolution = 0.16×0.16 mm, slice thickness = 2 mm, signal averages = 10-12.

The data from the last 30 min of each PET scan was used for high-definition (HD) PET imaging reconstruction, performed on the Siemens e7-tools software using point spread function corrected ordinary Poisson ordered subsets expectation maximization (PSF-OP–OSEM) algorithm with 21 subsets, 8 iterations, and a 2-mm full width at half maximum (FWHM) Gaussian smoothing filter. The size of the image matrices were 344×344×127 resulting in a pixel size of 1.04 mm and a slice thickness of 2.03 mm. MR-based 2-point Dixon attenuation correction for the PET image was also performed. Dynamic images were decay corrected to the injection time. An average PET spatial resolution of 4.3 mm at 1 cm offset from the center of field of view was expected to obtained from the PET/MR scanner (14).

Image analysis
PET images were co-registered with MR images using Inveon Research Workplace (IRW; Siemens Medical Solutions USA, Inc., Malvern, PA) to identify the location of the plaques developed in the femoral artery. The fused images were evaluated by measuring the radioactivity concentration within the regions of interests drawn on the injured femoral artery, sham-operated femoral artery and a representative area on the non-target background thigh muscle of each rabbit.

The mean and maximum of standardized uptake values (SUV\textsubscript{mean} and SUV\textsubscript{max}) of IF, SF, and BM were calculated by dividing the decay-corrected activity per unit volume of tissue (Bq/cm\textsuperscript{3}) by the injected activity per unit of body weight (Bq/g), as described by the following equation:

\[
\text{SUV} = \frac{\text{radioactivity concentration (Bq/cm}^3)}{\text{injected dose (Bq)/body weight (g)}}
\]

The mean cross-sectional area of the femoral artery blood vessel wall was measured in transverse MR images by subtracting the number of pixels of the inner blood vessel from the outside blood vessels in transverse MR images and taking the average of the slices containing the plaque.

**Histologic analysis**

The animals were injected intravenously with the hypoxia-reactive reagent pimonidazole hydrochloride (Hypoxprobe-1, Natural Pharmacia International, Belmont, MA) 1.5-2 h before euthanasia. The IF and SF were collected and perfusion-fixed with freshly prepared 3-4% paraformaldehyde solution to collect specimens for histopathological assessment. Specimens were embedded in
paraffin and sectioned transversely. To visualize the spatial co-localization of the hypoxic cells and macrophages, adjacent 5 µm thick cross-sections of the femoral artery were obtained.

IF and SF specimens were stained for the presence of hypoxia using the anti-pimonidazole antibody (Natural Pharmacia Inc., Belmont, MA) and mounted in solution containing 4'-6-diamidino-2-phenylindole (DAPI; Vector Laboratories, Palo Alto, CA) for fluorescent staining of nuclei. Although pimonidazole may react with reactive oxygen species, the anti-pimonidazole antibody only recognizes hypoxia derivatives (9). Fluorescein isothiocyanate was used as the secondary antibody.

Adjacent serial sections were assessed for macrophage content and hypoxia inducible factor-1α (HIF-1α) using mouse monoclonal antibodies for rabbit macrophages (clone RAM-11, Dako North America, Inc., Carpinteria, CA) and anti-HIF-1α (clone H1α67, Novus Biologicals, Littleton, CO).

The bound markers were detected by Cy3 and Cy2 secondary fluorescent antibodies, respectively. The slides were incubated with DAPI prior to coverslipping. Negative control staining was performed by replacing the primary antibody with a matching isotype control followed by the same fluorescent-labeled secondary antibody. Immunohistochemistry slides were imaged with use of a Leica TCS SP5 confocal laser scanning microscope.

**Statistical methods**
Data are presented as mean ± standard deviation. Statistical analysis was performed using GraphPad Prism 6 (GraphPad Sofrware, Inc., La Jolla, CA). Differences between the IF/BM and SF/BM SUV were assessed using the Student’s t-test. The correlation between the IF/SF femoral artery cross-section area ratios in MR images and the IF/SF SUV ratios in the corresponding ⁶⁴Cu-ATSM PET images were analyzed by simple linear regression with 95% confidence intervals. P < 0.05 was considered statistically significant.

RESULTS

PET/MR imaging showed increased uptake of ⁶⁴Cu-ATSM in the IF of all 5 rabbits 4 wk after injury, as indicated by the bright spots in both transverse and coronal views co-localizing to the lesion as identified by anatomic MRI (Fig. 2, A and D; Supplemental Fig. 1). Eight weeks post injury, significantly higher ⁶⁴Cu-ATSM was found in IF in the PET images fused with T₁-weighted MRI, T₂-weighted MRI and PD-weighted MRI (Fig. 2, B and E; Supplemental Fig. 2, PD-weighted MRI not shown). Higher uptake of ¹⁸F-FDG in the IF of the rabbits confirmed the development of advanced atherosclerotic-like plaque (Fig. 2, C and F; Fig. Supplemental Fig. 3).

The IF demonstrated 1.75 ± 0.21, and 2.30 ± 0.26 fold higher SUV<sub>mean</sub> ratios and 1.61 ± 0.15, and 1.84 ± 0.12-fold higher SUV<sub>max</sub> ratios 30-60 min p.i. of ⁶⁴Cu-ATSM in comparison to the SF 4 and 8 wk after injury (Supplemental Fig. 4), respectively. ¹⁸F-FDG imaging demonstrated 2.31 ± 0.29 IF/SF SUV<sub>mean</sub> ratios and
1.82 ± 0.26 IF/SF SUV\textsubscript{max} ratios 4 and 8 wk after injury (Supplemental Fig. 4).

IF/BM SUV ratios were significantly higher ($P < 0.001$) than SF/BM SUV ratios both 4 and 8 wk after injury p.i. of $^{64}$Cu-ATSM and 8 wk after injury p.i. of $^{18}$F-FDG ($P < 0.05$) (Fig. 3).

IF/SF SUV ratios measured from $^{64}$Cu-ATSM PET images did not show a significant correlation with specific signal on $T_1$, $T_2$, or PD-weighted MR images. Nevertheless, the combination of all three contrasts helped to delineate the plaque and lumen boundaries. The ratios of IF/SF blood vessel wall cross-sectional areas determined by $T_1$-weighted images was strongly positively correlated to IF/SF SUV\textsubscript{mean} ratios in a linear regression ($R^2 = 0.742$, $P = 0.0002$) (Supplemental Fig. 5). This finding suggested an increasing lipid content and hypoxic level in the IF during the progression of atherosclerosis, as supported by the thickening of the IF blood vessel wall.

Hematoxylin and eosin (H&E) stain demonstrated a focal thickened neointima comprised of foam cells and vascular smooth muscle cells was generated in the injured vessel (Supplemental Fig. 6). Immunohistochemical analysis showed that the induced atherosclerotic-like lesions contained macrophages as indicated by RAM-11, which co-localized to the HIF-1$\alpha$ positive area (Fig. 4, A, B and D; Supplemental Fig. 7). Hypoxia, as shown by pimonidazole staining of the adjacent section, was located in the deep macrophage-rich area within the atheromatous core of the plaque (Fig. 4, C). Superficial macrophages adjacent to the lumen stained negatively with pimonidazole, suggesting that these
superficial macrophages were not hypoxic, consistent with what has been described previously (15). No immunofluorescence of RAM-11, HIF-1α, or pimonidazole was seen in the sham-operated femoral artery sections (Supplemental Fig. 8).

DISCUSSION

Although ⁶⁴Cu-ATSM has been investigated for imaging tumor hypoxia in animal and human studies (16-18), its use in the assessment of cardiovascular disease has so far been limited. Retrospective review of PET-CT examinations in cervical cancer patients imaged with ⁶⁴Cu-ATSM at our institution revealed ⁶⁴Cu-ATSM uptake in regions corresponding to atherosclerosis (note: personal communication between Farrokh Dehdashti and Pamela K. Woodard). To our knowledge, this study is the first investigation of ⁶⁴Cu-ATSM as a hypoxic cell PET imaging agent in a non-murine animal model of atherosclerosis.

Although ¹⁸F-fluorodeoxyglucose (FDG), an agent used to image metabolic activity as a glucose analog, has also been used in atherosclerosis imaging (19) and has been shown to detect vascular atheroma (19), it provides limited value for evaluating the coronary arteries due to the confounding effects of myocardial uptake of the radiotracer. ⁶⁴Cu-ATSM imaging would overcome this limitation. ¹⁸F-FMISO is one of the most commonly used investigational PET agents for the measurement of tumor hypoxia (20). Recently, the feasibility of this PET imaging agent for in vivo detection of hypoxia in advanced lesions was successfully
evaluated in rabbits with advanced atherosclerosis (15). However compared to 

64Cu-ATSM, 18F-FMISO has two major disadvantages: lower cellular uptake and slower washout from normoxic tissue (20). The more efficient uptake and washout kinetics of 64Cu-ATSM in hypoxic and normoxic cells offers the possibility of a faster and more selective means of detecting hypoxia by PET imaging. 18F-EF5 has also been studied to detect hypoxic plaques in mice with atherosclerosis by ex vivo methods, but the slow blood clearance and high adventitial uptake limit its value for in vivo imaging of atherosclerosis (21). These findings suggest that 64Cu-ATSM has the potential to be a more discriminating agent for hypoxia in atherosclerosis assessment.

Although PET/CT as a hybrid scanner has in the past emerged as a valuable modality in clinical use as well as an important research tool, the fully integrated PET/MR scanners provide the excellent soft tissue contrast and functional imaging capabilities of MR (22). The described morphological features of plaque vulnerability can be nicely imaged by MRI (23) and the biological features of plaque vulnerability can be visualized by PET with different tracers and targets in a complementary fashion (24-26). Thus PET/MR allows for multiparametric profiling of plaque morphology and potentially vulnerability in one imaging session and should facilitate the identification of high-risk plaques in patients with atherosclerotic disease (22). These non-oncological applications may further benefit from lower radiation exposure of the patients in PET/MR compared to PET/CT (27). In light of these advantages, imaging of atherosclerosis using fully
integrated hybrid PET/MR yields an additional diagnostic value both over stand-alone PET and MR scanners and as well as over hybrid PET/CT.

The hematoxylin and eosin stain, along with our previous work (11), shows that a combination of a cholesterol-enriched diet and air dessication produce a focal thickened neointima in the area of air dessication comprised of foam cells and vascular smooth muscle cells. The duration of cholesterol diet in these animals is too short to see lesions induced in peripheral arteries outside of the region of air dessication, thus allowing us to use the contralateral sham artery as a control. The hypoxia-specific dye pimonidazole used for histopathologic characterization ex vivo is a 2-nitroimidazole containing a basic, piperidine moiety and reduces in cells with low oxygen tension. It is considered as the “gold standard” immunohistochemical hypoxia marker and widely used as a hypoxia-specific dye (pO2 ≤ 10 mm Hg). The resulting pimonidazole derivatives form protein adducts, which can be detected by immunostaining (28). This agent has been used primarily in oncologic specimen assessment (29) and only recently in assessment of atherosclerosis (9). The co-localization of the macrophage marker (RAM-11) and hypoxia indicators (pimonidazole) suggested the uptake of 64Cu-ATSM was associated with the presence of macrophages. Despite stimuli other than hypoxia that may induced HIF-1 in normoxia (30), a previous study (15) demonstrated that nearly all macrophages present in the plaque expressed HIF-1α, and a strong correlation between the hypoxic region (pimonidazole) and macrophage (RAM-11) density in plaque by immunohistology. Thus, the presence of HIF-1α in the same region as the macrophages in the lesions served as supportive evidence of
hypoxia in atherosclerotic macrophages. In addition, the finding that hypoxia was mainly located in the deep, macrophage-rich area within the atheromatous core is consistent with this study (15), which suggests that enough oxygen can diffuse from the lumen to nourish the shallow macrophage population but not the deep macrophage-rich core owing to the increased consumption and/or reduced supply of O\textsubscript{2} and nutrients from the lumen and vasa vasorum to those regions. The significantly higher \textsuperscript{18}F-FDG uptake in the injured femoral artery further helped to confirm the development of advanced plaque and accumulated macrophages in this region. In previous publications, it was shown that macrophages have a high uptake of \textsuperscript{18}F-FDG (31). Moreover, even though we did not assess the biodistribution of \textsuperscript{64}Cu-ATSM in blood, muscle, and femoral arteries to validate the complete clearance of radiopharmaceuticals at the time periods used for SUV calculation, our methods for SUV comparison – injured femoral to sham femoral SUV ratios – show the absence of radioactivity in the blood pool excluding potential bias that could result from the radioactivity contribution from the blood pool (31).

One limitation of our study is that the SUVs were not corrected for partial-volume effects (PVEs). The quantitative accuracy of PET is reduced by PVEs primarily due to the limited spatial resolution of the scanner leading to underestimation of the SUV with decreasing structural size of plaques (32,33). In practice, PVE is minimal when the dimensions of homogenous uptake region are > 2- to 3-times the spatial resolution of the scanner (34). The spatial resolution of the Siemens mMR PET/MR system is approximately 4.3 mm (14,35), while the rabbit’s femoral artery is 1.5-1.8 mm in diameter (36). As a result quantification of tracer
uptake in rabbit atherosclerotic plaque is likely to be significantly affected by PVEs. We are currently developing PVEs correction techniques to exploit the ability of PET/MR to provide high resolution PET images for improved absolute quantitative assessment of hypoxia in atherosclerotic plaque.

Furthermore, in this project, we used standard multi-contrast MRI sequences to image femoral arterial plaque with the initial intent of identifying plaque components in this rabbit model of atherosclerosis. However, because of the relatively small plaque size, limited MRI spatial resolution, and simple plaque components (smooth muscle cells and foam cells), it was difficult to consistently differentiate plaque components by this approach and to obtain quantitative plaque composition information from MR images to conduct specific studies correlating MRI characteristics of atherosclerosis with PET signal.

CONCLUSION

In summary, we demonstrate the ability of $^{64}$Cu-ATSM PET to noninvasively detect hypoxia in advanced atherosclerosis-like lesions in an animal model. $^{64}$Cu-ATSM PET/MR is a promising imaging method for detecting hypoxic and potentially vulnerable atherosclerosis in human subjects.

DISCLOSURE

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Washington University Institute of Clinical and Translational Sciences grant UL1TR000448 from the National Center for Advancing Translational Sciences (NCATS) of the National Institutes of Health (NIH), Department of Energy Grant DESC0002032, and NIH National Research Service Award (5-T32-HL07081-38) from the National Heart, Lung and Blood Institute. No other potential conflict of interest relevant to this article was reported.

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REFERENCE


13. Voller TF. Procedure: preparation of 64Cu-diacetyl-bi [N4-methylthiosemicarbazone](64Cu-ATSM). 2008, Washington University School of Medicine, St. Louis


FIGURE LEGENDS

Figure 1. Study protocol for imaging atherosclerosis rabbits.
Figure 2. The transverse (top) and coronal (bottom) view of $^{64}$Cu-ATSM PET/T1-weighted MR images of a representative rabbit 4 wk (A, D) and 8 wk (B, E) post injury, and $^{18}$F-FDG PET/ T$_1$-weighted MR images of the same rabbit 8 wk post injury (C, F). Red arrows point to injured femoral artery; blue arrows point to sham-operated femoral artery.
Figure 3. IF/BM SUV$_{\text{mean}}$ ratios were significantly higher than SF/BM SUV$_{\text{mean}}$ ratios 4 and 8 wk post injury in $^{64}$Cu-ATSM imaging, and 8 wk post injury in $^{18}$F-FDG imaging.
Figure 4. In the atherosclerotic plaque of the injured femoral artery, HIF-1α staining is localized within areas of high RAM-11-positive macrophages (A), confirmed by viewing single channels staining of RAM-11 (B) and HIF-1α (D). Detection of pimonidazole (PIMO) adducts was done on the adjacent slide (C), and pimonidazole positivity was observed in the area of RAM-11+HIF-1α displayed in panels A, B and D. Panels A and C are at the same scale. For all panels the color code is as follows: DAPI, white; RAM-11, red; HIF-1α, blue; PIMO, green.
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