PET imaging of focal demyelination and remyelination: comparison of $^{11}$CMeDAS, $^{11}$CIC and $^{11}$CPIB

Daniele de Paula Faria, Sjef C.V.M. Copray, Jurgen W.A. Sijbesma, Antoon T.M. Willemsen, Carlos A. Buchpiguel, Rudi A.J.O. Dierckx, Erik F.J. de Vries

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ABSTRACT

Recently, several candidate tracers for imaging of myelin loss with Positron Emission Tomography (PET) have been described. PET of myelin can facilitate monitoring of disease progression and evaluation of new treatment strategies. In this study, we compared the ability of \(^{11}\text{C}\)CIC, \(^{11}\text{C}\)MeDAS and \(^{11}\text{C}\)PIB to reveal temporal changes in myelin content in focal lesions in the lysolecithin rat model for multiple sclerosis. Pharmacokinetic modeling was performed to determine the best method to quantify tracer uptake. Sprague-Dawley rats were stereotactically injected with either 1% lysolecithin or saline in the corpus callosum and striatum of the right brain hemisphere. Dynamic PET imaging was performed 7 days after saline injection (control group) and 7 days and 4 weeks after lysolecithin injection (demyelination and remyelination group, respectively). \(^{11}\text{C}\)CIC, \(^{11}\text{C}\)MeDAS and \(^{11}\text{C}\)PIB could be better fitted by Logan graphical analysis, suggesting that tracer binding is reversible. Compartment modeling revealed that all tracers fitted best with the reversible two-tissue compartment model. All tracers were able to demonstrate demyelination and remyelination processes in the focal lesion. However, the slow kinetics and homogeneous brain uptake of \(^{11}\text{C}\)CIC make this tracer less suitable for \textit{in vivo} PET imaging. \(^{11}\text{C}\)PIB showed good uptake in white matter in cerebrum, but \(^{11}\text{C}\)PIB uptake in cerebellum was low, despite high myelin density in this region. In contrast, \(^{11}\text{C}\)MeDAS distribution correlates well with the myelin density in different brain regions. In conclusion, this study shows that PET imaging of demyelination and remyelination processes in focal lesions is feasible. Our comparison of three myelin tracers shows that \(^{11}\text{C}\)MeDAS has more favorable proprieties for quantitative PET imaging of demyelinated and remyelinated lesions throughout the CNS than \(^{11}\text{C}\)CIC and \(^{11}\text{C}\)PIB.
INTRODUCTION

Multiple Sclerosis (MS) is an inflammatory neurodegenerative disorder of the central nervous system. The disease is characterized by the formation of focal demyelinated lesions as a consequence of inflammatory damage to myelin and oligodendrocytes (Compston and Coles, 2008). Initially, lesions may recover when remyelination occurs. After multiple attacks in chronic progressive MS, however, remyelination fails and axons are left demyelinated and prone to degeneration (Franklin and Ffrench-Constant, 2008; Traveggia et al, 2010). MRI is frequently used for detection of lesions in MS. MRI measures differences in water concentration in tissue, but it is not specific for MS lesions, as differences in water concentration can be related to demyelination and remyelination, but also to edema, inflammation, gliosis and/or axonal loss (Phillipi and Rocca, 2011).

PET with ligands for myelin may be more specific for detecting ongoing demyelination and remyelination. Compounds with a stilbene structure like CIC and MeDAS, but also thiophene derivatives like PIB, have been explored as PET tracers for myelin imaging, as they were shown to bind to proteins with aggregated β-sheet structures. These β-sheet structures are also found in the myelin basic protein (MBP) when present in intact myelin. However, once myelin disintegrates (demyelination), the β-sheet structure of MBP is lost and consequently tracer binding decreases (Wu et al, 2006). [11C]CIC PET has been used for imaging myelin changes in the lysolecithin rat model (Wang et al, 2009), showing encouraging results. We have evaluated [11C]CIC in the cuprizone mouse model, but a poor correlation between ex vivo biodistribution and PET imaging was found. [11C]MeDAS PET could demonstrate differences in myelin content between transgenic hyper-myelinated and wild type mice (Wu et al, 2010). Recently, [11C]MeDAS was used to detect lesions in the spinal cord of lysolecithin...
injected mice and the experimental autoimmune encephalomyelitis model (Wu et al, 2013). $[^{11}\text{C}]$PIB is a widely used PET tracer for detection of β-amyloid plaques in Alzheimer’s disease. However, Stankoff et al (2011) recently showed that $[^{11}\text{C}]$PIB has affinity for white matter in animals and human sections and that $[^{11}\text{C}]$PIB was also able to detect focal lesions in two MS patients.

The aim of this study is to determine which tracer has the best characteristics for in vivo PET imaging of demyelinated lesions in MS. Therefore, we compared the imaging characteristics and kinetic properties of $[^{11}\text{C}]$CIC, $[^{11}\text{C}]$MeDAS and $[^{11}\text{C}]$PIB in the well-established lysolecithin-induced demyelination rat model for MS.

**MATERIAL AND METHODS**

**Animals**

Demyelination was induced in 8-10 weeks old male Sprague-Dawley rats (Harlan, the Netherlands) by unilateral stereotactic injection of 7 μl of a 1% lysolecithin (LPC; Sigma-Aldrich) solution in saline into the corpus callosum (3 μl) and striatum (4 μl) (anterior -0.30, lateral -3.0 and ventral -3.0 -4.2 -5.0 mm to Bregma point) at a speed of 0.1 μl/min. Controls were injected with saline, using the same procedure. The procedure was optimized to give 1.5 to 2.0 mm demyelinated lesions without major tissue damage (necrotic areas). Body weight and signs of movement disorders were monitored daily. Food and water were given ad libitum.

The animal experiments were performed according to the Dutch Regulations for Animal Welfare. The protocol was approved by the Institutional Animal Care and Use Committee of the University of Groningen (protocol: DEC 5040J).
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**Radiochemistry**

$^{11}\text{C}\text{MeDAS}$ was prepared as described by Wu et al (2010). The radiochemical purity was ≥95% and the specific activity >80 GBq/μmol. $^{11}\text{C}\text{CIC}$ was synthesized by the same procedure as $^{11}\text{C}\text{MeDAS}$. Exposure of $^{11}\text{C}\text{CIC}$ to light was avoided to prevent cis-trans isomerization. $^{11}\text{C}\text{CIC}$ had a radiochemical purity ≥95% and a specific activity >40 GBq/μmol. $^{11}\text{C}\text{PIB}$ was labeled as previously described (Solbach et al, 2005). Its radiochemical purity was >95% and the specific activity >50 GBq/μmol.

**PET imaging**

Animals were separated in 3 groups: 1) Control – animals injected with saline and imaged 1 week after stereotactic injection; 2) Demyelination – animals injected with 1% lysolecithin and imaged 1 week after stereotactic injection; 3) Remyelination – animals injected with 1% lysolecithin and imaged 4 weeks after injection. Each experimental group was evaluated with $^{11}\text{C}\text{CIC}$, $^{11}\text{C}\text{MeDAS}$ and $^{11}\text{C}\text{PIB}$ (each n = 6).

Rats were anesthetized with 2% isoflurane in medical air. A cannula was surgically inserted in the femoral artery for blood sampling and another cannula was placed in the femoral vein for tracer injection. Animals were positioned on their back in the microPET camera (Focus 220, Siemens) with their head in the field of view. Rats were injected in the femoral vein with 30-60 MBq of tracer in 1ml 10% ethanol by an infusion pump with a speed of 1 ml/min. Acquisition of a 60-min dynamic scan was started when the tracer entered the femoral vein. Animals were monitored during the whole scan procedure for heart rate and oxygen saturation. A transmission scan of 515 seconds with a $^{57}$Co point source was performed after the emission scan for the correction of attenuation and scatter.
Blood sample processing

Eighteen arterial blood samples (100 μl) were taken during the PET scan (at 1, 5, 10, 15, 20, 30, 45, 60, 75, 90, 120, 180, 300, 450, 600, 900, 1800, 3600 seconds). A larger blood sample (200 μl) was collected at 1, 5, 10, 15, 30, and 60 min, as these samples were also used for metabolite analysis. Heparinized saline was injected in the arterial cannula between blood samples to prevent large changes in blood pressure. A 25 μl aliquot of whole blood was taken for radioactivity measurement. In the remaining blood sample, plasma was separated from blood cells by centrifugation (5 minutes at 13000 rpm). Radioactivity in 25 μl plasma and 25 μl whole blood was measured with a gamma counter (LKB Wallac, Turku, Finland).

Metabolite analysis

Metabolite analysis of $[^{11}]$CPIB was performed by liquid-liquid extraction as described by Price et al (2005). The same procedure was used for metabolite analysis of $[^{11}]$CIC, except phosphate buffer pH 7.2 was used instead of ammonium formate buffer pH 4.2 due to the poor solubility of the carrier. Metabolite analysis of $[^{11}]$CMeDAS was performed by solid phase extraction. The plasma sample (100 μl) was diluted with 200 μl of 0.30 mol/L ammonium acetate (pH 10.0) and passed through an Oasis HLB 30 mg cartridge (Waters). The cartridge was washed with 0.5 ml 0.30 mol/L ammonium acetate (pH 10.0) and subsequently eluted with 1 ml acetonitrile. The radioactivity in the cartridge, aqueous and acetonitrile fractions were measured with a gamma counter. The percentage of intact tracer was calculated by dividing the activity in the acetonitrile fraction by the total amount of activity in the cartridge, the aqueous and the acetonitrile fractions.
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Each technique was validated by injecting the same plasma sample also into the HPLC quality control system of the respective tracer. The eluted fractions (1 fraction/min) were collected and counted with the gamma counter. The deviation between the extraction methods and HPLC analysis was <10%.

**PET image reconstruction and analysis**

The list-mode data of the emission scan was separated into 20 frames (8x30, 2x60, 2x120, 2x150, 3x300 and 3x600). Emission sinograms were iteratively reconstructed (OSEM2d, 4 iterations) after being normalized, corrected for attenuation and decay of radioactivity. Regions of interest (ROI) were drawn manually in different parts of the brain. To this purpose, the PET images were co-registered with a MRI template to facilitate the identification of the different brain regions. The exact position of the lesion was determined from the Luxol Fast Blue histochemistry images of the same animal and a ROI was drawn in the same region in the PET images. The ROI of the lesion was copied to the contralateral hemisphere for quantification of tracer uptake in the unaffected hemisphere. The results are presented as lesion-to-contralateral brain ratios.

The whole blood and plasma time-activity curves were corrected for decay and the plasma curve was also corrected for metabolites. Pharmacokinetic modeling and standardized uptake values (SUV) calculations were performed using INVEON research workstation software (Siemens). Graphical Logan analysis and Patlak analysis were used to determine the volume of distribution ($V_T$) and the metabolic rate ($K_i$). Subsequently, the one-tissue (1TCM) and the reversible two-tissue (2TCMR) compartment model were used to fit ROI data. The optimal model was selected based on the Akaike information criterion (AIC) values generated by the INVEON software.
Myelin histochemistry

After the PET procedure was completed, animals were terminated by intra-cardiac perfusion with 4% paraformaldehyde. Their brains were explanted and overnight submerged in 20% sucrose. Brains were cryosectioned in the coronal plane at 20 \( \mu \text{m} \) thickness. Sections containing the corpus callosum were dehydrated in an ascending ethanol series (50%, 70%, 80% and 96%) and incubated in the Luxol Fast Blue (LFB) solution (0.5 g Solvent Blue 38 (Sigma) in 500 ml 10% acetic acid in 96% ethanol) at 58 °C for 14-16 hours. After incubation, sections were washed with 96% ethanol and distilled water. Differentiation was performed in a 0.125% lithium carbonate solution, after which the sections were rinsed with 70% ethanol and distilled water, counterstained with cresyl violet and coverslipped with DePeX (Merck). Slides were analyzed with a Zeiss fluorescent microscope.

The extent of remyelination of the lesions was semi-quantitatively scored by an experienced observer, who was blinded for information about the experimental groups. A 4-point demyelination scale was used: (1) complete demyelination; (2) predominate demyelination (>50%) with small remyelinated areas; (3) small areas of demyelination (<50%) with remyelination in the major part of the lesion; (4) complete remyelination.

Statistical analysis

Results are presented as mean ± standard deviation. Differences between groups were analyzed for statistical significance by 2-way ANOVA (GraphPad Prism) using a Bonferroni post hoc test. Differences were considered significant when \( p<0.05 \).
RESULTS

Lysolecithin induced lesions
One week after lysolecithin injection demyelinated lesions, approximately 2 mm in diameter, were observed at the injection site. Four weeks after lysolecithin injection, lesion recovery was highly variable, indicating different remyelination rates between animals. No abnormalities were observed in the contralateral hemisphere of lysolecithin injected rats. The control group did not show any demyelination at all after saline injection. No differences in behavior, mobility or weight loss between control and lysolecithin-injected animals were noticed.

Tracer uptake in control rats
\[^{11}\text{C}]\text{CIC}\) was uniformly distributed throughout the brain in control rats (Figure 1). Highest tracer uptake (SUV 50-60 min) was found in midbrain (1.01±0.46) and thalamus (1.00±0.44). Other brain regions had uptake values between 0.86 and 0.99.

\[^{11}\text{C}]\text{MeDAS}\) exhibited higher whole brain uptake at all time points than \[^{11}\text{C}]\text{CIC}\) and \[^{11}\text{C}]\text{PIB}\). \[^{11}\text{C}]\text{MeDAS}\) and \[^{11}\text{C}]\text{PIB}\) were more heterogeneously distributed over the brain than \[^{11}\text{C}]\text{CIC}\). Highest \[^{11}\text{C}]\text{MeDAS}\) uptake was found in brain regions with high white matter density, like brainstem (1.46±0.23) and midbrain (1.40±0.19). Lowest uptake was observed in the cortex (0.92±0.19). \[^{11}\text{C}]\text{PIB}\) distribution corresponded less well to white matter density. The highest \[^{11}\text{C}]\text{PIB}\) uptake was found in the thalamus (0.81±0.45) and the hypothalamus (0.78±0.43), whereas the lowest SUV was observed in the cerebellum (0.34±0.23). \[^{11}\text{C}]\text{PIB}\) uptake in cerebellum was significantly (3-fold) lower than the uptake of \[^{11}\text{C}]\text{MeDAS}\) (1.06±0.14, \(p<0.01\)) and \[^{11}\text{C}]\text{CIC}\) (0.97±0.41, \(p<0.05\)). \[^{11}\text{C}]\text{PIB}\) also
showed significantly lower uptake in highly myelinated regions like brainstem (0.50±0.35 p<0.001) and midbrain (0.69±0.44 p<0.01) than \(^{[11]}\text{C}\)MeDAS (1.46±0.23 and 1.40±0.19). The standard deviations for \(^{[11]}\text{C}\)PIB uptake were much higher (deviation >50%) then for \(^{[11]}\text{C}\)MeDAS.

The time-activity curves (TAC, Figure 1A) reveal that \(^{[11]}\text{C}\)CIC has a different kinetic brain uptake profile than \(^{[11]}\text{C}\)MeDAS and \(^{[11]}\text{C}\)PIB. The TACs of \(^{[11]}\text{C}\)MeDAS and \(^{[11]}\text{C}\)PIB showed a fast peak uptake, followed by gradual tracer washout. \(^{[11]}\text{C}\)MeDAS showed a higher peak uptake than \(^{[11]}\text{C}\)PIB, but a similar clearance half-life (18.5 vs. 18.0 min). \(^{[11]}\text{C}\)CIC showed a much lower initial uptake, followed by a slow increase in uptake over time. This suggests that \(^{[11]}\text{C}\)CIC exhibited poorer brain penetration, but stronger binding than \(^{[11]}\text{C}\)MeDAS and \(^{[11]}\text{C}\)PIB.

![Figure 1](image)

**Figure 1**: \(^{[11]}\text{C}\)CIC, \(^{[11]}\text{C}\)MeDAS and \(^{[11]}\text{C}\)PIB uptake in the brains of control rats (n=6). (A) Whole brain time-activity curves. (B) Tracer uptake in the different brain regions, 50-60 min after tracer injection. Statistical differences are represented by * (p<0.05) when comparing \(^{[11]}\text{C}\)CIC and \(^{[11]}\text{C}\)PIB and by °(p<0.05), °°(p<0.01) and °°°(p<0.001) when comparing \(^{[11]}\text{C}\)MeDAS and \(^{[11]}\text{C}\)PIB.

**Plasma clearance and metabolism**

As shown in Figure 2, no significant differences in plasma clearance were found between the evaluated tracers. \(^{[11]}\text{C}\)CIC and \(^{[11]}\text{C}\)MeDAS showed similar metabolic
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rates, whereas $[^{11}\text{C}]$PIB was metabolized faster. Five minutes after injection, the percentage of parent tracer was 61±7%, 62±6% and 39±7% for $[^{11}\text{C}]$CIC, $[^{11}\text{C}]$MeDAS and $[^{11}\text{C}]$PIB respectively, whereas at 60 min these percentages decreased to 29±13%, 32±6% and 14±7%, respectively. Metabolites of $[^{11}\text{C}]$CIC, $[^{11}\text{C}]$MeDAS and $[^{11}\text{C}]$PIB were all more hydrophilic than the intact tracer, suggesting that metabolites are less likely to cross the blood brain barrier (BBB).

Figure 2: *Left:* plasma clearance of $[^{11}\text{C}]$CIC, $[^{11}\text{C}]$MeDAS and $[^{11}\text{C}]$PIB during the 60-min dynamic PET scan. The insert shows a magnification of the first 3 min of the curve. *Right:* The percentage of intact tracer in plasma from the time of injection until the end of the PET scan.

**PET imaging of lysolecithin induced lesions**

One week after lysolecithin injection, $[^{11}\text{C}]$CIC, $[^{11}\text{C}]$MeDAS and $[^{11}\text{C}]$PIB PET could all clearly detect focal demyelinated lesions (Figure 3A). Four weeks after lysolecithin injection, the degree of remyelination varied considerably between animals (*vide infra*) and likewise tracer uptake in the lesions showed a high degree of variability. No lesions were detected after saline injection. Moreover, clear delineation of the spinal cord was observed in the $[^{11}\text{C}]$MeDAS and $[^{11}\text{C}]$PIB images, but not in the $[^{11}\text{C}]$CIC images (Figure 3B).
Figure 3: (A) Illustrative $[^{11}\text{C}]$CIC (top row), $[^{11}\text{C}]$MeDAS (middle row) and $[^{11}\text{C}]$PIB (bottom row) PET images (last frame: 50-60 min) of control rats (left lane) and rats 1 week (middle lane) and 4 weeks (right lane) after lysolecithin injection. Arrows indicate the injection site. (B) Sagittal view of control rats. Arrows indicate the spinal cord.

Quantification of tracer uptake during demyelination and remyelination

Tracer uptake in the lesion was quantified as SUV and expressed lesion-to-contralateral brain ratios (Figure 4). The lesion-to-contralateral brain ratios for $[^{11}\text{C}]$CIC, $[^{11}\text{C}]$MeDAS and $[^{11}\text{C}]$PIB were close to unity in control animals (0.98±0.04, 0.97±0.005 and 0.97±0.05, respectively). In the demyelination group, all tracers showed a significantly reduced lesion-to-contralateral brain ratio (0.86±0.07, p<0.01; 0.90±0.03, p<0.001 and 0.81±0.04, p<0.001 for $[^{11}\text{C}]$CIC, $[^{11}\text{C}]$MeDAS and $[^{11}\text{C}]$PIB, respectively). Lesion-to-contralateral brain ratios in the remyelination group were still significantly lower than controls for $[^{11}\text{C}]$CIC (0.89±0.09, p<0.05) and $[^{11}\text{C}]$MeDAS (0.91±0.06, p<0.01), but not significantly different from the demyelination group. The lesion-to-contralateral brain ratio of $[^{11}\text{C}]$PIB in the remyelination group (0.97±0.04, p<0.01), however, was
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significantly higher than in the demyelination group and not significantly different from control animals anymore.

![Comparison of $[^{11}C]$CIC, $[^{11}C]$MeDAS and $[^{11}C]$PIB for focal demyelination PET imaging](image)

**Figure 4:** $[^{11}C]$CIC (left), $[^{11}C]$MeDAS (middle) and $[^{11}C]$PIB (right) lesion-to-contralateral brain uptake ratios (n=6). Significant differences with controls are illustrated by *** (p<0.001), ** (p<0.01) and * (p<0.05). Significant differences between the demyelination group and the remyelination group are indicated by ### (p<0.001).

**Kinetic modeling fitting**

None of the tracers showed any significant differences in plasma clearance and metabolism rate between controls and the demyelination and remyelination groups.

Tracer kinetics were analyzed by Logan and Patlak graph analysis, using delay times of 10 min and 20 min, respectively. Logan analysis showed a significantly better fit than Patlak analysis for $[^{11}C]$CIC (0.998±0.001 vs. 0.997±0.003; p=0.046), $[^{11}C]$MeDAS (0.999±0.0002 vs. 0.984±0.006; p=0.0003) and $[^{11}C]$PIB (0.999±0.0003 vs. 0.997±0.001; p=0.001). Compartment modeling was carried out using the 1TCM and 2TCMR. The AIC indicated that the 2TCMR gave the best fit for $[^{11}C]$CIC, $[^{11}C]$MeDAS and $[^{11}C]$PIB (Table 1).
Table 1: AIC for the fit of $^{11}$C[CIC, $^{11}$C]MeDAS and $^{11}$C]PIB PET data with 1TCM and the 2TCMR.

<table>
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<th></th>
<th>1TCM</th>
<th>2TCM</th>
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<td>449±15</td>
<td>401±32</td>
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</tr>
<tr>
<td>[C]MeDAS</td>
<td>494±12</td>
<td>457±21</td>
<td>p=0.010</td>
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<tr>
<td>[C]PIB</td>
<td>485±18</td>
<td>433±10</td>
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**Volume of distribution**

The $V_T$ was determined by 2TCMR and by Logan graph analysis (Figure 5). An excellent correlation between 2TMCR and Logan analysis was found for $^{11}$C]MeDAS ($r^2=0.98$) and $^{11}$C]PIB ($r^2=0.99$), whereas the correlation was substantially worse for $^{11}$C]CIC ($r^2=0.78$). The points on the $^{11}$C[CIC regression that showed the worst correlation were derived from small ROIs of the lesions in the demyelination and remyelination groups, suggesting that the 2TMCR for $^{11}$C]CIC is more sensitive to noise. Likewise, an excellent correlations between $V_T$ (Logan) and uptake (SUV) lesion-to-contralateral brain was also observed for $^{11}$C]MeDAS ($r^2=0.95$) and $^{11}$C]PIB ($r^2=0.94$), but not for $^{11}$C]CIC ($r^2=0.57$).
Comparison of $[^{11}\text{C}]$CIC, $[^{11}\text{C}]$MeDAS and $[^{11}\text{C}]$PIB for focal demyelination PET imaging

Figure 5: Correlation between $V_T$ determined by the 2TCMR and Logan graph analysis (top row) for (A) $[^{11}\text{C}]$CIC, (B) $[^{11}\text{C}]$MeDAS and (C) $[^{11}\text{C}]$PIB. Correlation between the $V_T$ ratio (Logan) and the lesion-contralateral brain uptake ratio (bottom row) for (D) $[^{11}\text{C}]$CIC, (E) $[^{11}\text{C}]$MeDAS and (F) $[^{11}\text{C}]$PIB.

None of the tracers showed any significant differences in $V_T$ (Logan) of the lesion between the control, demyelination and remyelination group: $4.01\pm1.23$, $3.13\pm1.47$ and $2.64\pm0.98$ for $[^{11}\text{C}]$CIC; $3.28\pm0.90$, $3.93\pm1.03$ and $3.62\pm0.84$ for $[^{11}\text{C}]$MeDAS and $3.39\pm0.45$, $4.75\pm0.61$ and $4.11\pm1.40$ for $[^{11}\text{C}]$PIB, respectively.

To reduce inter-subject variability, lesion-to-contralateral brain $V_T$ ratios were calculated (Figure 6). The $V_T$ (Logan) lesion-to-contralateral brain ratios of $[^{11}\text{C}]$CIC (0.73±0.09), $[^{11}\text{C}]$MeDAS (0.90±0.03) and $[^{11}\text{C}]$PIB (0.81±0.05) in the demyelination group were significantly lower ($p<0.001$) than in control rats (1.02±0.10, 0.98±0.01 and 0.98±0.04, respectively). The $V_T$ ratio of $[^{11}\text{C}]$CIC in the remyelination group (0.85±0.11) was significantly higher ($p<0.05$) than in the demyelination group, but still significantly lower ($p<0.001$) than in control animals, suggesting partial remyelination. The $V_T$ ratio of $[^{11}\text{C}]$MeDAS in the
remyelination group (0.90±0.05) was not significantly different from the demyelination group, indicating absence of remyelination. In contrast, $V_T$ ratio of $[^{11}\text{C}]$PIB in the remyelination group (0.96±0.04) was similar to control animals and significantly higher ($p<0.001$) than in the demyelination group, suggesting complete remyelination.

When the entire hemisphere was considered, no differences between the $V_T$ of the left and right hemisphere were found for any of the tracers, indicating that the myelin changes were restricted to the focal lesion.

**Figure 6**: $[^{11}\text{C}]$CIC (left), $[^{11}\text{C}]$MeDAS (middle) and $[^{11}\text{C}]$PIB (right) $V_T$ (Logan) lesion-to-contralateral brain ratios (n=6). Significant differences compared to controls are represented by *** ($p<0.001$). Significant differences between demyelination and remyelination groups are indicated by # ($p<0.05$) and ### ($p<0.001$).

**Myelin histochemistry**

Myelin histochemistry revealed no abnormalities in control animals, whereas all lysolecithin-treated rats presented focal demyelination 1 week after injection. Four weeks after lysolecithin injection, animals showed highly variable myelin recovery. Surprisingly, hardly any remyelination was observed in the $[^{11}\text{C}]$MeDAS group (1.6±1.3), whereas intermediate and almost complete remyelination was found in the $[^{11}\text{C}]$CIC group (2.6±1.1) and $[^{11}\text{C}]$PIB group (3.3±0.8), respectively.
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Figure 7: LFB histochemistry: (A) Control rat showing no demyelination in corpus callosum and striatum; (B) Demyelination in the corpus callosum and striatum 1 week after lysolecithin injection; (C) Demyelination in the corpus callosum 4 weeks after lysolecithin injection; (D) Demyelination in the striatum 4 weeks after lysolecithin injection; (E) Incomplete remyelination in the corpus callosum and striatum 4 weeks after lysolecithin injection; (F) Complete remyelination 4 weeks after lysolecithin injection. cc = corpus callosum, str = striatum, ctx = cortex, LV = lateral ventricle. Scale bar = 200 μm.

DISCUSSION

$[^{11}\text{C}]\text{CIC}$, $[^{11}\text{C}]\text{MeDAS}$ and $[^{11}\text{C}]\text{PIB}$ have previously been evaluated as tracers for PET imaging of myelin changes (Wang et al, 2009; Wu et al, 2010; Wu et al, 2013; Stankoff et al, 2011), but no direct comparison has been made so far. The present study demonstrates that these PET tracers can all reflect the myelin content in focal lesions in the lysolecithin rat model.

$[^{11}\text{C}]\text{PIB}$ is usually used for PET imaging of β-amyloid deposition in Alzheimer’s disease (Price et al, 2005; Klunk et al 2004), but the tracer also showed high uptake in white matter (Fodero-Tavolett et al, 2009). Stankoff and coworkers (2011) showed that $[^{11}\text{C}]\text{PIB}$ PET was able to detect lesions in an MS animal model and MS patients, suggesting that the tracer binds specifically to myelin. Kinetic modeling studies in healthy controls and Alzheimer patients showed that $[^{11}\text{C}]\text{PIB}$ kinetics were fitted best by the 2TCMR; the $V_T$ determined by Logan graph
analysis gave most reliable and stable results (Price et al, 2005). Our study confirmed that $[^{11}\text{C}]$PIB uptake can best be analyzed with the 2TCMR or Logan analysis. These modeling methods also worked best for $[^{11}\text{C}]$MeDAS and $[^{11}\text{C}]$CIC. Binding potentials calculated by the 2TCMR showed high variability for all 3 tracers, likely due to the lack of accuracy in $k_3$ and $k_4$ (Lammertsma, 2007). The $V_T$ (Logan) and $V_T$ (2TCMR) correlated well for $[^{11}\text{C}]$MeDAS and $[^{11}\text{C}]$PIB. The correlation was less strong for $[^{11}\text{C}]$CIC, especially for the small ROIs of the lesions, suggesting that the 2TCMR is more sensitive to noise for this tracer. Thus, $V_T$ (Logan) seemed to give the most stable results. Yet, high variability in $V_T$ was observed between individual animals, even in the control group. To decrease variability among animals, we used the ratio between the tracer uptake at the injection site and uptake in the same region in the contralateral hemisphere to measure changes in the tracer uptake during demyelination and remyelination. Interestingly, the $V_T$ ratio and the uptake (SUV) ratio correlated strongly for $[^{11}\text{C}]$MeDAS and $[^{11}\text{C}]$PIB indicating that a static PET scan without arterial blood sampling could give similarly reliable results as kinetic modeling.

All tracers were able to detect loss of myelin in the demyelination group, but analysis of the PET imaging results in the remyelination group seemed to suggest that only $[^{11}\text{C}]$PIB was able to detect remyelination. However, myelin histochemistry in the remyelination group showed that almost complete remyelination occurred only in the $[^{11}\text{C}]$PIB group, whereas intermediate remyelination was observed in the $[^{11}\text{C}]$CIC group and hardly any remyelination in the $[^{11}\text{C}]$MeDAS group. The same trend was also observed in the $V_T$ ratios of the different PET tracers. This means that the $V_T$ ratio of all tracers actually correctly reflects the extent of remyelination. However, other properties than lesion
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detection should also be considered when comparing the tracers for in vivo PET imaging.

A disadvantage of $^{11}\text{C}$CIC is the necessity to avoid light exposure during radiosynthesis to prevent the cis-trans isomerization of the tracer. Besides, $^{11}\text{C}$CIC shows low, homogeneous brain uptake and slow washout kinetics. The slow kinetics may indicate some level of irreversible binding, which is supported by the fact that $^{11}\text{C}$CIC performed better in the Patlak analysis than the other two tracers. Finally, $^{11}\text{C}$CIC does not show any uptake in the spinal cord, which would preclude imaging of lesions in the spinal cord.

Both $^{11}\text{C}$PIB and $^{11}\text{C}$MeDAS have an easy synthesis procedure and show faster brain clearance kinetics. Both tracers are able to visualize the spinal cord, but they show different distribution patterns in the brain. $^{11}\text{C}$MeDAS showed higher brain uptake than $^{11}\text{C}$PIB, especially in white matter regions. Lesions in MS patients can be found throughout the central nervous system, but the optic nerve, brainstem, cerebellum and spinal cord are sites of predilection (McDonald and Ron, 1999). $^{11}\text{C}$MeDAS uptake in (healthy) cerebellum and brainstem was significantly higher than $^{11}\text{C}$PIB, which makes $^{11}\text{C}$MeDAS the preferred tracer for lesion imaging. Price et al (2005) proposed the use of cerebellum as a reference tissue for kinetic modeling of $^{11}\text{C}$PIB in Alzheimer patients, due to the absence of plaques in cerebellum. The low uptake in cerebellum indeed suggests low specific binding to white matter in this region. In multiple sclerosis the low cerebellar $^{11}\text{C}$PIB uptake would complicate the detection of lesions in the cerebellum, since the demyelinated lesions show decreased signal compared to intact myelin.

Although $^{11}\text{C}$PIB seems to be less favorable than $^{11}\text{C}$MeDAS for MS lesion imaging, it is already available for clinical use. The study by Stankoff et al (2011) showed that $^{11}\text{C}$PIB can show lesions in patients, but it did not evaluate lesions
in the cerebellar region. We propose that additional studies in MS patients are needed to assess the feasibility of $^{11}$CPIB PET in imaging lesions throughout the brain and spinal cord, including the cerebellar region. In addition, studies using $^{11}$CMeDAS in patients are needed to confirm the promising results of this tracer in preclinical studies.

CONCLUSION

This study shows that $^{11}$CIC, $^{11}$CMeDAS and $^{11}$CPIB were all able to reveal demyelination in focal lesions of the lysolecithin injected rat model. Their kinetics showed to fit best to the two-tissue reversible compartment model. The lesion-to-contralateral $V_T$ ratio, either calculated by the 2TCMR or Logan analysis, appeared to be a reliable method to quantify myelin changes by PET imaging. However, the strong correlation between the $V_T$ ratio and the uptake ratio for $^{11}$CMeDAS and $^{11}$CPIB, makes the SUV the preferred parameter, as it requires a simpler procedure without blood sampling and metabolite analysis. The brain distribution of $^{11}$CMeDAS correlates better with white matter density in different brain regions than $^{11}$CIC and $^{11}$CPIB. Therefore, $^{11}$CMeDAS would be the preferred tracer to monitor changes in myelin density in white matter.

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