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Semiquantification Study of [^{11}C]-(*R*)-PK11195 PET Brain Images in Multiple Sclerosis

Estudo da Semiquantificação de Imagens PET Cerebrais de 11C]-(R)-PK11195 na Esclerose e Múltipla

Lucas D. L. Narciso[1], Phelipi N. Schuck[1], Caroline M. Dartora[1], Cristina S. Matushita[2], Jefferson Becker[2], Ana M. Marques da Silva[1,2]

[1]*Núcleo de Pesquisa em Imagens Médicas/Faculdade de Física/PUCRS, Porto Alegre, Brasil*
[2]*Instituto do Cérebro do Rio Grande do Sul (InsCer)/PUCRS, Porto Alegre, Brasil*

Resumo
Imagens PET cerebrais adquiridas com [11C]-(R)-PK11195 são utilizadas em estudos da ativação microglial, como na esclerose múltipla (EM). O objetivo deste estudo é investigar o comportamento da captação nas regiões justacortical e periventricular de [11C]-(R)-PK11195 em imagens PET reformatadas em diferentes intervalos temporais, através da aplicação de três métodos, buscando o método e intervalo temporal que melhor diferenciam pacientes com EM dos indivíduos saudáveis. Métodos semiquantitativos de SUV (Standardized Uptake Value) e de normalização da captação para região de referência (SUVR) foram aplicados a imagens PET, de diferentes intervalos temporais, adquiridas de 10 pacientes com EM e 5 indivíduos saudáveis. Identificou-se diferença estatisticamente significativa entre grupos nas regiões justacortical e periventricular dos valores de SUV ($p = 0,01$) no intervalo de 40 a 60 min, e no SUVR com a substância branca como referência ($p < 0,01$) para o intervalo de 10 a 60 min.
Palavras-chave: [11C]-(R)-PK11195; PET; esclerose múltipla; semiquantificação; SUV; SUVR.

Abstract
*PET brain images with [^{11}C]-(*R*)-PK11195 are being widely used to visualize microglial activation in vivo in neurodegenerative diseases, such as multiple sclerosis (MS). The aim of this study is to investigate the uptake behavior in juxtacortical and periventricular regions of [^{11}C]-(*R*)-PK11195 PET brain images reformatted in different time intervals by applying three methods, seeking method and time interval that significantly differentiate MS patients from healthy controls. Semiquantitative SUV and SUVR methods were applied to PET images from different time intervals acquired from 10 patients with MS and 5 healthy controls. The results show significant difference between groups for SUV ($p = 0.01$) in 40 to 60 min interval, and SUVR with white matter as reference ($p < 0.01$) in 10 to 60 min interval, measured in the juxtacortical and periventricular regions.*
Keywords*: [^{11}C]-(*R*)-PK11195; PET; multiple sclerosis; semi-quantification; SUV, SUVR.*

1. Introduction

Positron Emission Tomography (PET) and Magnetic Resonance (MR) imaging have been widely used in neurodegenerative brain diseases[1,2].

Multiple Sclerosis (MS) is a neurodegenerative disease with important social impact by affecting mostly young-adults, although a low prevalence disease. Nowadays, the diagnosis is based on a 2010 revision from McDonald's criteria[3], in which one criterion is the presence of one or more lesions in two of the four characteristic regions (juxtacortical, periventricular, infratentorial and spinal cord).

Microglial activation occurs in a significant way after important neuronal damages, such as results from degenerative processes[4]. The compound PK11195 is an important choice to activated microglia *in vivo* imaging with PET, when ^{11}C radiolabeled, indicating active inflammatory disease with a wide applicability[4].

Activity concentration normalization to a reference region (such as cerebellum) or the simplified reference tissue model[5] have been used in most quantitative studies of [^{11}C]-(*R*)-PK11195 PET brain images, obtaining reasonably accurate binding potential values even without arterial blood sampling[6–9].

Semiquantification has been proposed as simpler analysis methods, based on region of interest (ROI) activity concentration normalization to a reference region (SUVR). Among those, the method proposed by Debruyne et al. (2002) consider the whole brain mean activity concentration from the last time frame as reference[10]. Such choice resulted in accurate data to normal volunteers[10,11]. Another similar method was proposed by Hammoud et al. (2005), which considers the white matter mean concentration activity as reference[12], which resulted in increased [^{11}C]-(*R*)-PK11195 specific binding in positive HIV patients' images[12].

Semiquantitative [^{11}C]-(*R*)-PK11195 methods are applied to static images from a time interval from the whole acquisition, usually in list mode.

However, there is no consensus on which time interval should be used to apply these methods. Diverse time intervals have been presented, such as:

- 5 to 20 min, period with higher accumulated radiotracer activity and best image quality, excluding perfusion phase[13];
- 5 to 30 min, time interval in which pseudo-equilibrium is reached in supposedly high density areas of activated microglia[14];
- 40 to 60 min, period in which time-activity curves presented similar decline in steady state for both patients and control subjects (40 min post injection)[10,15];
- Other studies also used 0 to 60 min[16], 10 to 60 min[12] and 30 to 60 min[9] time intervals.

Interpretations of the semiquantitative methods results have been limited to relate increased [^{11}C]-(*R*)-PK11195 uptake areas to known pathology and its distribution[17].

The aim of this study is to investigate the uptake behavior in juxtacortical and periventricular regions of [^{11}C]-(*R*)-PK11195 PET brain images reformatted in different time intervals by applying three semiquantitative methods (SUV, $SUVR_{WM}$ e $SUVR_{Deb}$), seeking method and time interval that significantly differentiate MS patients from healthy controls.

2. Materials and Methods

PET [^{11}C]-(*R*)-PK11195 and MR brain images were acquired from volunteers of the project "Microglial activation assessment by PET/CT and Magnetic Resonance lesion appearance association in relapsing-remitting Multiple Sclerosis patients under fingolimoid treatment" [*our translation*], CAAE 23949813.7.0000.5336, approved in June 06, 2015, approval number 1.094.228.

Volunteers were divided into two groups: five from the control group [(24 ± 2) years old, minimum 20 years old and maximum 28 years old, and (74 ± 10) kg] and ten diagnosed with relapsing-remitting MS [(28 ± 3) years old, minimum 18 years old and maximum 35 years old, and (67 ± 15) kg].

Both acquisitions (PET e MRI) happened on the same day. The mean [^{11}C]-(*R*)-PK11195 administered activity to acquire the PET images was (560 ± 100) MBq [(15 ± 3) mCi, 390 MBq to 740 MBq]. The Centre for Radiopharmaceuticals Production produced the radiotracer, from the Brain Institute of Rio Grande do Sul from PUCRS.

Acquisitions were in list mode in a GE Healthcare PET/CT equipment, model Discovery 600, with BGO detector crystals. Images were reconstructed with 300 mm field of view, using VUE Point HD iterative algorithm, smoothing filter cutoff frequency 4.0 mm, 32 subsets and two iterations. The images' matrix have 192x192 pixels, 16-bits per pixel (0.640 pixels/mm resolution), pixel size of 1.56x1.56 mm^2 and 47 axials slices of 3.27 mm thickness each. Attenuation was corrected using a computed tomography image acquired previously to the PET acquisition. Other quantification needed corrections were applied (normalization, decay, scatter and random detections).

MR images were acquired on a 3.0 T GE Healthcare Signa HDxt equipment. Structural T1-weigthed MR images were acquired using BRAVO^{TM} sequence (brain volume), from GE Healthcare, with 2400 ms repetition time; 16 ms eco time; 220 mm field of view; with 1 mm isotropic voxels. T1-weigthed MR images present 512x512x196 pixels matrix (240.03x240.03x196.00 mm^3), 16-bits per pixel, 2.133 pixels/mm resolution, 0.47x0.47x1.00 mm^3 voxel size, and 6.12 ms frame interval.

2.1. PET Images Reformatting

List mode acquired PET images were reformatted as a mean image from some time intervals:

(A) 0 - 60 min[16] (whole acquisition);
(B) 5 - 20 min[13] (15 min, after perfusion);
(C) 5 - 30 min[14] (25 min, after perfusion);
(D) 10 - 60 min[12] (last 50 min);
(E) 30 - 60 min[9] (last 30 min); e
(F) 40 - 60 min[10,15] (last 20 minutes).

Previously segmented gray matter (GM) and white matter (WM) regions were used as masks to data extraction from juxtacortical and periventricular regions. The following figure shows an example of mask used to data extraction.

Figure 1. Example of mask used to PET images data extraction, in which the regions of interest are shown in white (juxtacortical and periventricular).

2.2. Pre-processing

PET and MR images were co-registered using SPM8 (http://www.fil.ion.ucl.ac.uk/spm/). MR images were GM, WM and cerebrospinal fluid regions segmented with SPM8. All MR images were corrected for equipment's magnetic field inhomogeneities[18] using FSL[19].

2.3. Semiquantitative Analysis

Standardized uptake values (SUVs) were obtained from all PET images and its results compared with the activity concentration normalization methods (SUVR), both applied on juxtacortical and periventricular regions. The SUVR methods are described below:

- Debruyne *et al*. (2002): activity concentration normalization to whole brain last time frame activity concentration ($SUVR_{Deb}$)[10]; and

- Hammoud *et al*. (2005): activity concentration normalization to WM activity concentration ($SUVR_{WM}$)[12].

Free software ImageJ (http://imagej.nih.gov/ij/) was used to data extraction.

2.4. Statistical Analysis

Differences between groups (patients with MS and controls) were evaluated using Student *t* test. Data normality was tested with the Shapiro-Wilk test using SPSS version 17.0.0 (SPSS, Heverlee, Belgium). Values are expressed as mean ± one standard deviation.

Spearman's rank correlation coefficient was used in EDSS (Expanded Status Scale Kurtzke Inability), disease duration, number of relapses and age, to a correlation check with the semiquantitative values from the methods previously described in this study.

After initial statistical analysis, outliers were identified using block diagrams and excluded.

3. Results

The following tables present the obtained SUV (Table 1), $SUVR_{Deb}$ (Table 2) and $SUVR_{WM}$ (Table 3) values.

SUV values in juxtacortical and periventricular regions indicate that the best time interval to differentiate MS patients from health control is (F), the last 20 minutes of the list mode acquisition (p = 0.09). Mean SUV values in this case is (0.37 ± 0.11) g/mL to MS patients and (0.29 ± 0.07) g/mL to health control.

Table 1. SUV mean values and standard deviation for MS patients (P) and health control group (C). The last table lines present the statistical tests *t*-values and *p*-values.

	SUV (g/mL)					
Time interval	**(A) 0 - 60 min**	**(B) 5 - 20 min**	**(C) 5 - 30 min**	**(D) 10 - 60 min**	**(E) 30 - 60 min**	**(F) 40 - 60 min**
	Whole acquisition	15 min, after perfusion	25 min, after perfusion	Last 50 min	Last 30 min	Last 20 min
Mean P	0.62 ± 0.19	0.63 ± 0.18	0.59 ± 0.17	0.48 ± 0.14	0.39 ± 0.12	0.37 ± 0.11
Mean C	0.49 ± 0.14	0.49 ± 0.17	0.45 ± 0.15	0.37 ± 0.11	0.30 ± 0.08	0.29 ± 0.07
Ratio P/C	1.29 ± 0.54	1.30 ± 0.59	1.30 ± 0.58	1.31 ± 0.55	1.30 ± 0.51	1.30 ± 0.49
***t*-value**	1.584	1.490	1.542	1.689	1.799	1.848
***p*-value**	0.15	0.18	0.16	0.13	0.10	0.09

Table 2. $SUVR_{Deb}$ mean values and standard deviation for MS patients (P) and health control group (C). The last table lines present the statistical tests *t*-values and *p*-values.

	$SUVR_{Deb}$ (unitless)					
Time interval	**(A) 0 - 60 min**	**(B) 5 - 20 min**	**(C) 5 - 30 min**	**(D) 10 - 60 min**	**(E) 30 - 60 min**	**(F) 40 - 60 min**
	Whole acquisition	15 min, after perfusion	25 min, after perfusion	Last 50 min	Last 30 min	Last 20 min
Mean P	1.72 ± 0.21	1.74 ± 0.10	1.61 ± 0.08	1.31 ± 0.04	1.08 ± 0.02	1.02 ± 0.02
Mean C	1.68 ± 0.19	1.67 ± 0.29	1.55 ± 0.24	1.26 ± 0.14	1.05 ± 0.06	1.00 ± 0.03
Ratio P/C	1.02 ± 0.17	1.04 ± 0.19	1.04 ± 0.17	1.04 ± 0.12	1.03 ± 0.06	1.02 ± 0.04
***t*-value**	0.368	0.521	0.582	0.814	1.127	1.344
***p*-value**	> 0.20	> 0.20	> 0.20	> 0.20	> 0.20	> 0.20

Table 3. $SUVR_{WM}$ mean values and standard deviation for MS patients (P) and health control group (C). The last table lines present the statistical tests *t*-values and *p*-values.

	$SUVR_{WM}$ (unitless)					
Time interval	**(A) 0 - 60 min**	**(B) 5 - 20 min**	**(C) 5 - 30 min**	**(D) 10 - 60 min**	**(E) 30 - 60 min**	**(F) 40 - 60 min**
	Whole acquisition	15 min, after perfusion	25 min, after perfusion	Last 50 min	Last 30 min	Last 20 min
Mean P	1.11 ± 0.03	1.09 ± 0.04	1.07 ± 0.03	1.03 ± 0.02	0.99 ± 0.02	0.98 ± 0.02
Mean C	1.15 ± 0.02	1.13 ± 0.02	1.12 ± 0.02	1.07 ± 0.02	1.03 ± 0.02	1.02 ± 0.03
Ratio P/C	0.97 ± 0.03	0.96 ± 0.03	0.96 ± 0.03	0.96 ± 0.03	0.96 ± 0.03	0.96 ± 0.03
***t*-value**	-2.757	-3.114	-3.421	-3.807	-3.351	-3.005
***p*-value**	0.02	< 0.01	< 0.01	< 0.01	0.02	0.03

$SUVR_{Deb}$ results (Table 2) in juxtacortical and periventricular regions did not result in statistically significant difference between MS patients and controls, in none of the chosen time intervals (p > 0.20).

$SUVR_{WM}$ results (Table 3) in juxtacortical and periventricular regions results in a statistically significant difference between MS patients and control group for all chosen time intervals. Within the analyzed time intervals, intervals (B) to (D) presented statistically significant difference with p-value < 0.01. Interval (D), last 50 minutes of the acquisition, obtained the most significant difference (t = -3.807, p < 0.01).

Mean $SUVR_{WM}$ in PET images juxtacortical and periventricular regions for interval (D) was (1.03 ± 0.02) to MS patients group and

(1.07 ± 0.02) to healthy control group (approximately 4% higher).

After the initial results evaluation, outliers were identified using block diagrams and removed. The following table (Table 4) shows the changed values after the outliers' removal.

Table 4. Statistical tests *t*-values and *p*-values for SUV and $SUVR_{WM}$ methods results after outliers removal.

SUV (g/mL)		
Time interval	***t*-value**	***p*-value**
(D) 10 - 60 min	3.014	0.01
(E) 30 - 60 min	3.032	0.01
(F) 40 - 60 min	2.975	0.01
$SUVR_{WM}$		
(B) 5 - 20 min	-2.800	0.02
(C) 5 - 30 min	-3.096	0.01
(D) 10 - 60 min	-3.608	< 0.01

After removing the outliers (one control), SUVs results in juxtacortical and periventricular regions begin to indicate a statistically significant difference ($p = 0.01$). Mean SUV values from (F) time interval to MS patients changed to (0.37 ± 0.11) g/mL and (0.26 ± 0.03) g/mL for the control group. There was no significant difference in the results of $SUVR_{WM}$ after the removal of outliers (one control in time intervals B and C, and one control and one MS patient in time interval D).

Statistically significant correlation was found between age and $SUVR_{WM}$ for (A) PET image ($p = 0.05$). No other statistically significant correlation was found.

4. Discussion

SUV values analysis results indicate that the images closer to the steady state period (after 40 min of radiotracer injection) present most statistically significant difference ($p = 0.09$), although the desired level of significance ($p = 0.05$) had not been reached at this point of the study. After the outliers' removal, however, the significance level became $p = 0.01$.

There are similar SUV values from the ones obtained in the study of Kumar et al. (2012)[13]. The same study also reports the correlation between age and SUV values[13] identified in this study ($p = 0.05$).

Applying $SUVR_{Deb}$ method did not lead to satisfactory results for juxtacortical and periventricular ROIs in this study and the comparison between the groups was not statistically significant ($p > 0.20$) for any of the time intervals, even when the outliers were removed.

Finally, the results of $SUVR_{WM}$ indicate that there is a statistically significant difference between groups for the juxtacortical and periventricular regions of [^{11}C]-(*R*)-PK11195 PET images for all time intervals. Among these time intervals, results extracted from the image obtained by the mean activity concentration from 10 to 60 min of the acquisition presented the most significant difference between the groups ($t = -3.807$, $p < 0.01$). This indicates that $SUVR_{WM}$ presents better results when the image contains both counts from the beginning and the end of the acquisition.

5. Conclusions

$SUVR_{WM}$ semiquantitative method applied in juxtacortical and periventricular regions in [^{11}C]-(*R*)-PK11195 PET brain images allows statistically significant difference between MS patients and control group. The most significant difference between the groups occurs by applying this method on the mean PET image obtained from the time interval 10 to 60 minutes from the acquisition in list mode.

PET images SUV values also allow differentiation between MS patients and control group, particularly when the image is obtained from the last 20 minutes acquisition mean.

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Contact:
Lucas Diovani Lopes Narciso
Av. Ipiranga, 6681, Pr. 96A sala 220, Partenon, Porto Alegre, RS. CEP 90619-900.
E-mail: lucas.narciso@acad.pucrs.br