MOLECULAR IMAGING AND BIOLOGY

Published Online: 10 April 2010

Mol Imaging Biol (2011) 13:10–15
DOI: 10.1007/s11307-010-0324-y

BRIEF ARTICLE

Reduced PBR/TSPO Expression After Minocycline Treatment in a Rat Model of Focal Cerebral Ischemia: A PET Study Using [^{18}F]DPA-714

Abraham Martín,[1] Raphaël Boisgard,[1] Michael Kassiou,[2] Frédéric Dollé,[3] Bertrand Tavitian[1]

[1]*CEA, DSV, I²BM, SHFJ, Laboratoire Imagerie Moléculaire Expérimentale; INSERM U803, 4, Place Général Leclerc, 91406, Orsay Cedex, France*
[2]*Department of Medical Radiation Sciences and School of Chemistry, University of Sydney, Sydney, NSW, Australia*
[3]*CEA, DSV, I²BM, SHFJ, Orsay, France*

Abstract

Background: Many new candidate pharmaceuticals designed to improve recovery after stroke have been proposed recently, but there are still too few molecular imaging methods capable to assess their efficacy. A hallmark of the inflammatory reaction that follows focal cerebral ischemia is overexpression of the mitochondrial peripheral benzodiazepine receptor/18 kDa translocator protein (PBR/TSPO) in the monocytic lineage and astrocytes. This overexpression can be imaged with positron emission tomography (PET) using PBR/TSPO-selective radioligands such as [^{18}F]DPA-714.
Purpose: Here, we tested whether PET with [^{18}F]DPA-714 would evidence the effect of minocycline, a broad spectrum antibiotic presently tested as neuroprotective agent after stroke, on the inflammatory reaction induced in an experimental model of stroke.
Procedures: Ten rats were subjected to a 2-h transient middle cerebral artery occlusion with reperfusion. Minocycline or saline was intravenously administrated 1 h after reperfusion and daily during the following 6 days. PET studies were performed using [^{18}F]DPA-714 at 7 days after cerebral ischemia.
Results: In vivo PET imaging showed a significant decrease in [^{18}F]DPA-714 uptake at 7 days after cerebral ischemia in rats treated with minocycline with respect to saline-treated animals. Minocycline treatment had no effect on the size of the infarcted area.
Conclusion: Minocycline administered daily during 7 days after ischemia decreases [^{18}F]DPA-714 binding, suggesting that the drug exerts an anti-inflammatory activity. [^{18}F]DPA-714 PET is a useful biomarker to study novel anti-inflammatory strategies in experimental cerebral ischemia.

Key words: Minocycline, PET, Neuroinflammation, PBR, TSPO, DPA-714, Cerebral ischemia

Introduction

Stroke remains a leading cause of adult disability and the major cause of death in the population worldwide. Currently, thrombolysis using recombinant tissue plasminogen activator (rTPA) is the only pharmacologic treatment approved for the acute phase of ischemic stroke, but safety issues restrict its use to the first 3 h after the onset of a nonhemorrhagic stroke, leaving more than 90% of stroke patients outside its indications [1]. Therefore, neuroprotective drugs appear as a promising

Correspondence to: Bertrand Tavitian; *e-mail:* Bertrand.tavitian@cea.fr

therapy for the management of stroke, especially if they could be administered at late after stroke onset. One of the important pathophysiological mechanisms involved during the acute phase of stroke is neuroinflammation that has been associated with an increase of infarct size and a worse clinical outcome [2]. Several anti-inflammatory strategies have been proposed in order to decrease the ischemic damage [3]. Recently, minocycline, a semisynthetic tetracycline, showed a powerful anti-inflammatory effect in animal models of global and permanent ischemia [4–7] inhibiting the activation of microglial cells [8, 9]. Activation of the monocytic lineage (microglia and infiltrating macrophages) is characterized by a dramatic increase in the expression of the peripheral benzodiazepine receptor/18 kDa translocation protein (PBR/TSPO) [10] after transient cerebral ischemia in rodents [11, 12]. In contrast, under physiological conditions brain parenchyma shows low expression levels of PBR/TSPO, making this mitochondrial protein an attractive target for *in vivo* imaging of cerebral inflammation [13, 14]. We have recently evaluated [^{18}F]DPA-714, a new imaging radioligand, in experimental middle cerebral artery occlusion (MCAO) in rat, and shown that it provides accurate quantitative information of PBR/TSPO expression. Positron emission tomography (PET) and autoradiography with [^{18}F]DPA-714 showed a time-dependent increase in tracer uptake in the ischemic lesion compared to the unaffected brain region [12]. [^{18}F]DPA-714 has also been evaluated *ex vivo* in striatal lesions in rat brains, and *in vivo*; in healthy baboons [15] and in herpes encephalitis [16] and acute neuroinflammation in rats [17], and is presently undergoing clinical trials.

Here, we evaluated [^{18}F]DPA-714 as an *in vivo* surrogate markers for monitoring the effect of minocycline treatment on the PBR/TSPO expression induced by neuroinflammation after cerebral ischemia in rats.

Materials and Methods

Animals and Surgery

Animal studies were approved by the animal ethics committee and conducted in accordance with the Directives of the European Union on animal ethics and welfare. Transient focal ischemia was induced by a 2-h intraluminal occlusion of the middle cerebral artery (MCA) followed by reperfusion, in adult male Sprague–Dawley rats (300 g body weight; Charles River, France; n=10) as described elsewhere [18]. Briefly, rats were anesthetized with 4% isofluorane in 100% O_2. A 2.6-cm length of 4-0 monofilament nylon suture that had been heat-blunted at the tip was introduced into the right external carotid artery up to the level where the MCA branches out. After occlusion, anesthesia was discontinued, and rats allowed recovering. After 2 h, the animals were re-anesthetized, the filament was removed, and the clip on the common carotid artery was released to allow reperfusion. Animals were studied at 7 days after reperfusion following the episode of ischemia.

Radiochemistry

DPA-714 (*N*,*N*-diethyl-2-(2-(4-(2-fluoroethoxy)phenyl)-5,7-dimethylpyrazolo[1,5-a]pyrimidin-3-yl)acetamide) was labeled with fluorine-18 (half-life, 109.8 min) at its 2-fluoroethyl moiety using a tosyloxy-for-fluorine nucleophilic aliphatic substitution according to slight modifications of procedures already reported [15]. This simple one-step process has been automated on our Zymate-XP robotic system [19] and then implemented on a commercially available GE TRACERLab FX-FN synthesizer. The process involves (A) reaction of K[^{18}F]F-Kryptofix®222 with the tosyloxy precursor (4.5–5.0 mg, 8.2–9.1 μmol) at 165°C for 5 min in DMSO (0.6 mL) followed by (B) C-18 PrepSep cartridge pre-purification and finally (C) semi-preparative HPLC purification on a Waters X-Terra™ RP18. Final formulation of [^{18}F]DPA-714 as an *i.v.* injectable solution (physiological saline containing less than 10% of ethanol) was performed using a home-made SepPak®Plus C18 device. Typically, 5.6–7.4 GBq of [^{18}F]DPA-714 (>95% chemically and radiochemically pure) was routinely obtained with specific radioactivities ranging from 37 to 111 GBq/μmol within 85–90 min (HPLC purification and SepPak®-based formulation included), starting from a 37 GBq [^{18}F]fluoride batch (overall non decay-corrected and isolated radiochemical yield: 15 to 20%).

PET Scans and Data Acquisition

PET studies were performed 7 days after induction of cerebral ischemia. Anesthesia was induced with 4% isofluorane and maintained by 2–2.5% of isofluorane in 100% O_2. During imaging the rat's head was placed in a home-made stereotaxic frame compatible with PET acquisition, and rats were maintained normothermic using a heating blanket (Homeothermic Blanket Control Unit, Harvard Apparatus Limited, Edenbridge, Kent, UK). The tail vein was catheterized with a 24-gauge catheter for intravenous administration. PET imaging was performed on the SIEMENS Concorde Focus 220 camera dedicated to small animal imaging. 74.1 MBq of [^{18}F]DPA-714 were injected concomitantly with the start of the PET acquisition.

Emission sinograms were normalized, corrected for attenuation and radioactive decay, and reconstructed using FORE and OSEM 2D (16 subsets and 4 iterations). PET images with [^{18}F]DPA-714 were reconstructed in 17 dynamic consecutive frames.

Image Analysis

PET images were co-registered to the anatomical data of a MRI rat brain template [20] in order to verify the anatomical location of the signal. Regions of interest (ROI) were manually defined for each rat on the region of increased binding in the ipsilateral hemisphere, and a spherical ROI containing the major part of the territory irrigated by the middle cerebral artery was defined in the homologous contralateral hemisphere. The mean value of each ROI in the PET images was measured using Anatomist software. Values were expressed in% of injected dose per cubic centimeter (ID/cc) for each ROI.

Minocycline Administration and Evaluation of Brain Damage

Rats were treated with either vehicle (saline; n=5) or minocycline hydrochloride (Sigma; n=5), administered intravenously 1 h after MCAO at a dose of 10 mg/kg followed by a daily dose (10 mg/kg) during the following 6 days. Rats were imaged by PET with [^{18}F]DPA-714, 7 days after reperfusion. Immediately after the PET scan,

rats were sacrificed, their brain removed and sliced in 2-mm-thick sections that were stained with 2,3,5-triphenyltetrazolium chloride (TTC; Sigma-Aldrich) for 10 min at 37°C. Infarct volumes (pale areas) were measured after staining with an image analysis system (AIS; Imaging Research Inc).

Statistical Analyses

Differences between control and treated animals were evaluated by the unpaired *t* test.

Results

[18F]DPA-714 Positron Emission Tomography after MCAO

An increase in [18F]DPA-714 binding was detected with PET at day 7 after ischemia in the stroke area (ipsilateral) with respect to the contralateral area (Fig. 1A). The kinetics of the [18F]DPA-714 PET signal was measured in the region of infarct (ipsilateral) and in the contralateral symmetrical (control) region (see Methods) at 7 days after induction of cerebral ischemia. The time–activity curves showed that, in the lesion, radioactivity uptake reached a peak rapidly a few minutes after the bolus injection of [18F]DPA-714 maintaining similar values for the next 30 min. In the control area, the peak uptake of radioactivity was immediate (1 min post [18F]DPA-714) but followed by a washout (Fig. 1B). Uptake in the ischemic hemisphere at 30 min after [18F]DPA-714 showed a 30% reduction of in animals treated with minocycline, statistically significant ($P<0.05$) with respect to the control animals (Fig. 1C). The volume of infarction showed a small (<10%), nonstatistically significant trend to decrease in animals treated with minocycline compared to control animals (Fig. 1D).

Discussion

The goal of this study was to evaluate the ability of PET imaging with [18F]DPA-714 to monitor *in vivo* anti-inflammatory actions of minocycline in a rat model of cerebral ischemia. Previous studies have shown a perfect correlation in [18F]DPA-714 binding over time between *in vivo* PET imaging and *ex vivo* autoradiography [12]. This correlation was sufficiently convincing to support the use of [18F]DPA-714 as a surrogate imaging marker for anti-inflammatory strategies in experimental cerebral ischemia in the present study. PBR/TSPO is considered as an attractive and sensitive marker for the quantification and visualization of the inflammatory processes presented in cerebral ischemia. Expression of PBR/TSPO is barely detected in the intact rodent brain but readily increases during neuroinflammation. Therefore, the microglial response to injury, such as migration, proliferation, and phagocytosis, may be related to PBR/TSPO upregulation after brain injury [21]. Recent immunohistochemistry studies also showed a steady increase of PBR/TSPO expression in microglia/macrophages, with a maximum at 11 days after transient MCA occlusion, followed by a trend to return to normal values of activated microglia at 30 days [12]. Remarkably, [18F]DPA-714 uptake over time was mainly associated with this increase in PBR/TSPO expression shown by immunohistochemistry [12]. Because late inflammation is known to significantly contribute to the outcome after an ischemic insult, reducing late inflammation represents a possible target for novel therapeutic agents. Detection of high levels of PBR/TSPO at some distance in time from initial ischemia suggests that it is relevant to this perspective. Several compounds have been proven to reduce brain damage after cerebral ischemia in animals, and studies have demonstrated the neuroprotective benefit of minocycline in a wide range of acute neurological injuries, such as focal cerebral ischemia [5, 22], transient global brain ischemia [4], spinal cord injury [23], and intracranial hemorrhage [24, 25]. Minocycline is a second-generation tetracycline that exerts anti-inflammatory effects inhibiting activated ameboid-shape of microglia at 24 h after a rat model of transient cerebral ischemia [5]. Minocycline has multiple effects in brain injury preventing caspase-1 and 3 activated apoptosis [26], cyclooxigenase 2 [4], inducible nitric oxide synthase [4, 5], p38 mitogen-activated protein kinase [27, 28], and matrix metalloproteinase-9 (MMP-9) [22] activity.

In the present study, we imaged [18F]DPA-714 uptake with PET at 7 days after cerebral ischemia and demonstrate a significant decrease of PBR/TSPO expression induced by treatment with minocycline. Our previous study showed that the maximal level of PBR/TSPO expression was observed at 11 days after ischemia, but that at this time point, there was also an increase in [18F]DPA-714 binding in the contralateral area [12]. This increase in the control area may reflect the recruitment of resident microglia at distance from the ischemic site, perhaps through low-grade expansion of neural impairment by spreading depression [29] or other mechanisms. Whatever the case, we chose to monitor the animals at 7 days after ischemia, before the increase in [18F]DPA-714 uptake in control areas can be observed [12]. At this time point in the ischemic area, [18F]DPA-714 showed a fast uptake during the first minutes after the injection and remained at plateau level from 30 to 60 min afterwards, as already shown in previous studies [12]. In the control area, [18F]DPA-714 uptake decreased rapidly due to the low expression presented in the non-ischemic hemisphere. This supports the use of 30 min PET scans (0–30 min after [18F]DPA-714 injection) uptake after minocycline treatment in stroked animals.

As shown by [18F]DPA-714 uptake, a daily intravenous dose of 10 mg/kg minocycline reduces significantly the expression of PBR/TSPO in the ischemic area at 7 days after a transient 2-h middle cerebral artery occlusion. Other studies have demonstrated neuroprotective effects using high intraperitoneal doses of minocycline (up to 90 mg/kg)

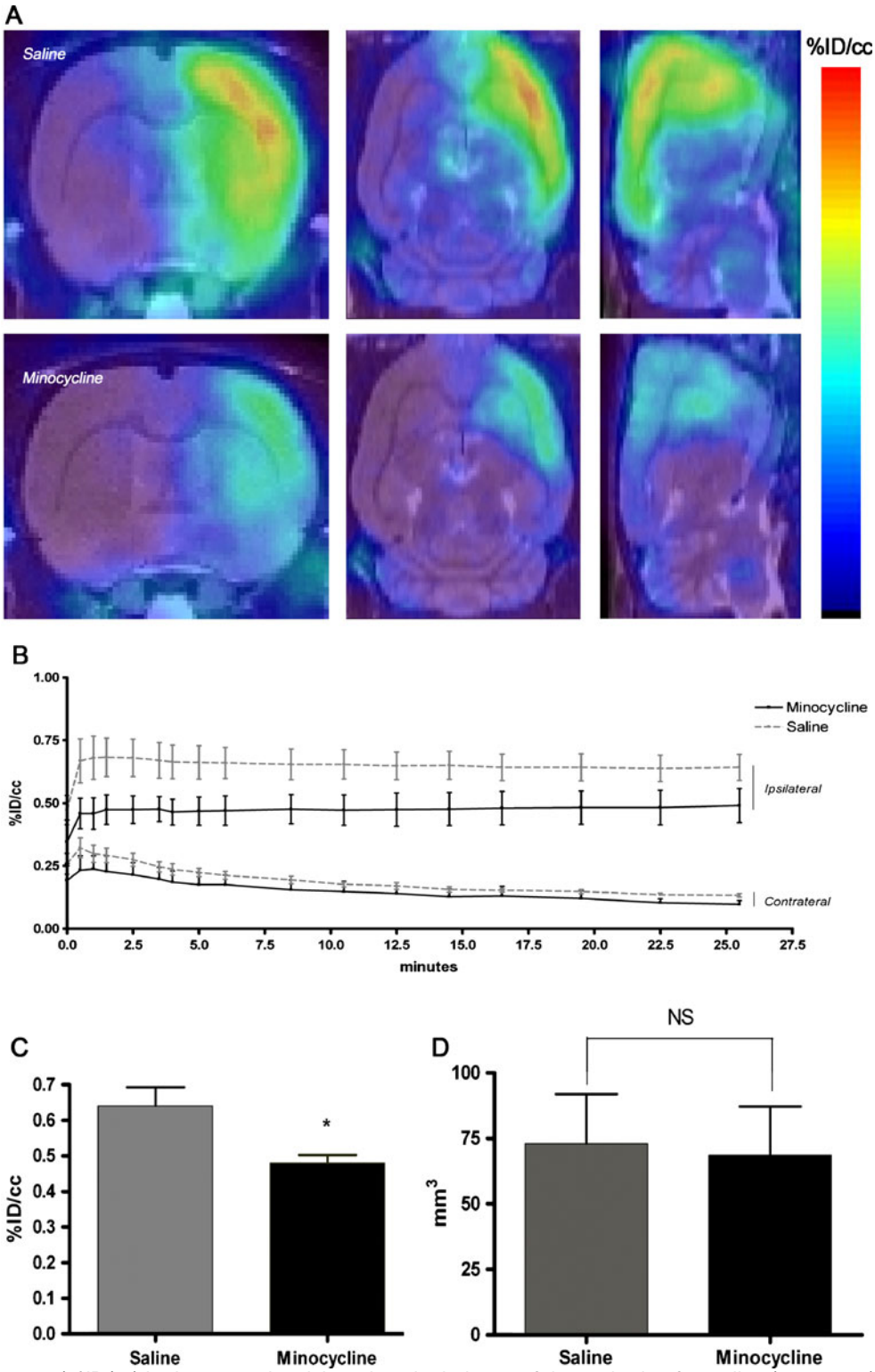

Fig. 1. **A** PET images (%ID/cc) in the coronal, axial, and sagittal planes of the rat brain after saline (*upper row*) and minocycline (*lower row*) treatment at 7 days after cerebral ischemia. Summed images of [^{18}F]DPA-714 (0–30 min) were normalized for radioactivity concentrations according to the scale shown on the *right*, and coregistered with a MRI rat brain atlas for illustration of anatomical regions. **B** Time–activity curves of [^{18}F]DPA-714 in rats treated with saline (*full line*, $n=5$) or minocycline (*dotted line*, $n=5$). Percent of injected dose per cubic centimeter (%ID/cc; mean±SD) was quantified in two ROIs defined in the region of infarction and a contralateral uninfarcted area. **C** Means±SD radioactivity uptake 30 min after [^{18}F]DPA-714 administration PET in the infarcted area in rat brains of treated and control rats 7 days after ischemia. Uptake decreases significantly in minocycline treated animals (*black box*; $n=5$) versus saline-treated (*gray box*; $n=5$) animals. *$P<0.05$. **D** Means±SD volume of infarction 7 days after cerebral ischemia in minocycline treated animals (*black box*; $n=5$) versus saline-treated (*gray box*; $n=5$) animals. *NS* $P>0.05$.

in models of global [4] and focal [5, 9] cerebral ischemia in rodents. The intravascular route therefore appears more efficient than the intraperitoneal route for minocycline delivery to the brain [30]. It also has the advantage to avoid the stress response linked to abdominal irritation after repeated intraperitoneal administration [31]. Xu et al. reported that "low" doses of intravenous minocycline induced a reduction of the infarct size and an amelioration of the neurological deficits, 24 h after a 90-min transient middle cerebral occlusion in rats [7]. In contrast, 7 days after cerebral ischemia, we did not observe a significant decrease in infarct volume in the minocycline treatment group, despite the decrease observed in [18F]DPA-714 uptake at the same time point. The difference between the results of our and Xu et al.'s study likely reveals differences in the observation time in the two studies, i.e. 1 and 7 days post-ischemia for Xu's and our present study, respectively. Moreover, it has been hypothesized that the inhibition by minocycline of matrix metalloproteinases (MMPs) after severe cerebral ischemia may have opposite effects at different times: immediately after ischemia, MMP activity mediates pathological reactions whose inhibition by minocycline has an early beneficial effect [7, 24, 25]. In contrast, at later times during the neuroinflammatory response, MMPs contribute to beneficial processes such as neuroplasticity, vascular and functional recovery, and the inhibition of their activity by minocycline would have a deleterious effect [32]. Finally, it is important to recognize that the "low" doses administered in both studies are still three to four times higher than the average 200 mg daily dosage of minocycline prescribed in humans for other indications [33]. Even though rodents often require higher doses because of a higher rate of liver metabolism, other dose-effect and dose-time-post-stroke studies remain to be conducted. Accordingly, [18F]DPA-714 has the capacity to monitor the effects of future therapeutic approaches on secondary inflammation after cerebral ischemia.

Acknowledgments. We thank A. Blossier for technical assistance in the PET studies. This work was funded in part by the EU-FP6 network EMIL (LSHC-CT-2004-503569) and the EU-FP6 network DiMI (LSHB-CT-2005-512146).

References

1. The National Institute of Neurological Disorders and Stroke rt-PA Stroke Study Group (1995) Tissue plasminogen activation for acute ischemic stroke. N Engl J Med 333:1581-1587
2. Montaner J, Rovira A, Molina CA, Arenillas JF, Ribo M, Chacon P, Monasterio J, Alvarez-Sabin (2003) Plasmatic level of neuroinflammatory markers predict the extent of diffusion-weighted image lesions in hyperacute stroke. J Cereb Blood Flow Metab 23:1403-1407
3. Emsley HC, Tyrrell PJ (2002) Inflammation and infection in clinical stroke. J Cereb Blood Flow Metab 22:1399-419
4. Yrjänheikki J, Keinänen R, Pellikka M, Hökfelt T, Koistinaho J (1998) Tetracyclines inhibit microglial activation and are neuroprotective in global brain ischemia. Proc Natl Acad Sci U S A 95:15769-15774
5. Yrjänheikki J, Tikka T, Keinänen R, Goldsteins G, Chan PH, Koistinaho J (1999) A tetracycline derivative, minocycline, reduces inflammation and protects against focal cerebral ischemia with a wide therapeutic window. Proc Natl Acad Sci U S A 96:13496-13500
6. Wang CX, Yang T, Shuaib A (2003) Effects of minocycline alone and in combination with mild hypothermia in embolic stroke. Brain Res 963:327-329
7. Xu L, Fagan SC, Waller JL, Edwards D, Borlongan CV, Zheng J, Hill WD, Feuerstein G, Hess DC (2004) Low dose intravenous minocycline is neuroprotective after middle cerebral artery occlusion-reperfusion in rats. BMC Neurol 26:4-7
8. He Y, Appel S, Le W (2001) Minocycline inhibits microglial activation and protects nigral cells after 6-hydroxydopamine injection into mouse striatum. Brain Res 909:187-193
9. Wu DC, Jackson-Lewis V, Vila M, Tieu K, Teismann P, Vadseth C, Choi DK, Ischiropoulos H, Przedborski S (2002) Blockade of microglial activation is neuroprotective in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine mouse model of Parkinson disease. J Neurosci 22:1763-1771
10. Papadopoulos V, Baraldi M, Guilarte TR, Knudsen TB, Lacapère JJ, Lindemann P, Norenberg MD, Nutt D, Weizman A, Zhang MR, Gavish M (2006) Trends Pharmacol Sci 27:402-409
11. Rojas S, Martín A, Arranz MJ, Pareto D, Purroy J, Verdaguer E, Llop J, Gómez V, Gispert JD, Millán O, Chamorro A, Planas AM (2007) Imaging brain inflammation with [11C]PK11195 by PET and induction of the peripheral-type benzodiazepine receptor after transient focal ischemia in rats. J Cereb Blood Flow Metab 27:1975-1986
12. Martín A, Boisgard R, Thézé B, Van Camp N, Kuhnast B, Damont A, Kassiou M, Dollé F, Tavitian B (2010) Evaluation of the PBR/TSPO radioligand [18F]DPA-714 in a rat model of focal cerebral ischemia. J Cereb Blood Flow Metab 30(1):230-241
13. Dollé F, Luus C, Reynolds A, Kassiou M (2009) Radiolabelled molecules for imaging the translocator protein (18 kDa) using Positron Emission Tomography. Curr Med Chem 16:2899-2923
14. Chaveau F, Boutin H, Van Camp N, Dollé F, Tavitian T (2008) Nuclear imaging of neuroinflammation: a comprehensive review of [11C]PK11195 challengers. Eur J Nucl Med Mol Imag 35:2304-2319
15. James ML, Fulton RR, Vercoullie J, Henderson DJ, Garreau L, Chalon S, Dolle F, Costa B, Guilloteau D, Kassiou M (2008) DPA-714, a new translocator protein-specific ligand: synthesis, radiofluorination, and pharmacologic characterization. J Nucl Med 49:814-822
16. Doorduin J, Klein HC, Dierckx RA, James M, Kassiou M, de Vries EF (2009) [11C]DPA-713 and [18F]DPA-714 as new PET tracers for TSPO: a comparison with [11C]-(R)-PK11195 in a rat model of herpes encephalitis. Mol Imaging Biol 11:386-398
17. Chauveau F, Van Camp N, Dollé F, Kuhnast B, Hinnen F, Damont A, Boutin H, James M, Kassiou M, Tavitian B (2009) Comparative evaluation of the translocator protein radioligands [11C]DPA-713, [18F]DPA-714, and [11C]PK11195 in a rat model of acute neuroinflammation. J Nucl Med 50:468-476
18. Justicia C, Martín A, Rojas S, Gironella M, Cervera A, Panés J, Chamorro A, Planas AM (2006) Anti-VCAM-1 antibodies did not protect against ischemic damage either in rats or in mice. J Cereb Blood Flow Metab 26:421-432
19. Damont A, Hinnen F, Kunhast B et al. (2008) Radiosynthesis of [18F]DPA-714, a selective radioligand for imaging the translocator protein (18 kDa) with PET. J Labelled Comp Radiopharm 51:286-292
20. Schweinhardt P, Fransson P, Olson L, Spenger C, Andersson JL (2003) A template for spatial normalisation of MR images of the rat brain. J Neurosci Methods 129:105-113
21. Ji B, Maeda J, Sawada M, Ono M, Okauchi T, Inaji M, Zhang MR, Suzuki K, Ando K, Staufenbiel M, Trojanowski JQ, Lee VM, Higuchi M, Suhara T (2008) Imaging of peripheral benzodiazepine receptor expression as biomarkers of detrimental versus beneficial glial responses in mouse models of Alzheimer's and other CNS pathologies. J Neurosci 28:12255-12267
22. Koistinaho M, Malm TM, Kettunen MI, Goldsteins G, Starckx S, Kauppinen RA, Opdenakker G, Koistinaho J (2005) Minocycline protects against permanent cerebral ischemia in wild type but not in matrix metalloprotease-9-deficient mice. J Cereb Blood Flow Metab 25:460-467
23. Stirling DP, Khodarahmi K, Liu J, McPhail LT, McBride CB, Steeves JD, Ramer MS, Tetzlaff W (2004) Minocycline treatment reduces delayed oligodendrocyte death, attenuates axonal dieback, and improves functional outcome after spinal cord injury. J Neurosci 24:2182-2190
24. Power C, Henry S, Del Bigio MR, Larsen PH, Corbett D, Imai Y, Yong VW, Peeling J (2003) Intracerebral hemorrhage induces macrophage activation and matrix metalloproteinases. Ann Neurol 53:731-742

25. Murata Y, Rosell A, Scannevin RH, Rhodes KJ, Wang X, Lo EH (2008) Extension of the thrombolytic time window with minocycline in experimental stroke. Stroke 39:3372-3377
26. Chen M, Ona VO, Li M, Ferrante RJ, Fink KB, Zhu S, Bian J, Guo L, Farrell LA, Hersch SM, Hobbs W, Vonsattel JP, Cha JH, Friedlander RM (2000) Minocycline inhibits caspase-1 and caspase-3 expression and delays mortality in a transgenic mouse model of Huntington disease. Nat Med 6:797-801
27. Du Y, Ma Z, Lin S, Dodel RC, Gao F, Bales KR, Triarhou LC, Chernet E, Perry KW, Nelson DL, Luecke S, Phebus LA, Bymaster FP, Paul SM (2001) Minocycline prevents nigrostriatal dopaminergic neurodegeneration in the MPTP model of Parkinson's disease. Proc Natl Acad Sci U S A 98:14669-14674
28. Tikka TM, Vartiainen NE, Goldsteins G, Oja SS, Andersen PM, Marklund SL, Koistinaho J (2002) Minocycline prevents neurotoxicity induced by cerebrospinal fluid from patients with motor neurone disease. Brain 125:722-731
29. Jander S, Schroeter M, Peters O, Witte OW, Stoll G (2003) Cortical spreading depression induces proinflammatory cytokine gene expression in the rat brain. J Neurosci 23:11602-11610
30. Fagan SC, Edwards DJ, Borlongan CV, Xu L, Arora A, Feuerstein G, Hess DC (2004) Optimal delivery of minocycline to the brain: implication for human studies of acute neuroprotection. Exp Neurol 186:248-251
31. Nessler S, Dodel R, Bittner A, et al (2002) Effect of minocycline in experimental autoimmune encephalomyelitis. Ann Neurol 52:689-690
32. Rosell A, Lo EH (2008) Multiphasic roles for matrix metalloproteinases after stroke. Curr Opin Pharmacol 8:82-89
33. Yong VW, Wells J, Giuliani F, Casha S, Power C, Metz LM (2004) The promise of minocycline in neurology. Lancet Neurol 3:744-751