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Effects of Translocator Protein (18 kDa) Ligands on Microglial Activation and Neuronal Death in the Quinolinic-Acid-Injected Rat Striatum

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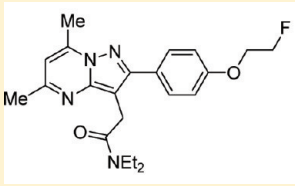
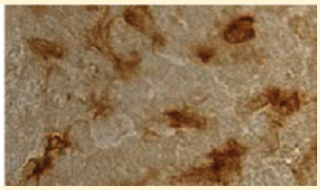
ABSTRACT: There is evidence that excitotoxicity and prolonged microglial activation are involved in neuronal death in neurodegenerative disorders. Activated microglia express various molecules, including the translocator protein 18 kDa (TSPO; formerly known as the peripheral benzodiazepine receptor) on the outer mitochondrial membrane. The TSPO is a novel target for neuroprotective treatments which aim to reduce microglial activation. The effect of PK 11195 and three other TSPO ligands on the level of microglial activation and neuronal survival was evaluated in a quinolinic acid (QUIN) rat model of excitotoxic neurodegeneration. All three ligands were neuroprotective at a level comparable to PK 11195. All of the ligands decreased microglial activation following the injection of QUIN but had no effect on astrogliosis. Interestingly, we also observed neuroprotective effects from the vehicle, dimethyl sulfoxide (DMSO).





KEYWORDS: *Neuroprotection, translocator protein, pyrazolopyrimidine, quinolinc acid, neuroinflammation, dimethyl sulfoxide*

Excitotoxicity and microglial activation are thought to be involved in neuronal death in a range of pathologies and neurodegenerative disorders.[1,2] Excitotoxicity occurs due to the overstimulation of *N*-methyl-D-aspartate (NMDA) receptors by glutamate and leads to increased calcium influx and production of free radicals which can trigger a cascade of events ultimately leading to necrotic or apoptotic neurodegeneration.[3,4] Quinolinic acid (QUIN) is an NMDA receptor agonist commonly used to model excitotoxic neurodegeneration. The unilateral intrastriatal injection of QUIN has been described as an animal model of Huntington's disease[5,6] and has been shown to produce large lesions accompanied by an inflammatory response involving increased microglial activation and proliferation.[7]

Microglia are the resident immune cells of the brain which, under normal conditions, display a ramified morphology with small cell bodies and long, thin processes. However, following pathological changes in the brain such as excitotoxic injury, they can become activated and rapidly change morphology, adopting an amoeboid-like shape. This change in morphology in the activated state is accompanied by the release of pro-inflammatory cytokines and high levels of the excitotoxins QUIN and glutamate which can cause further neuronal damage.[8] Therefore, it has been proposed that prolonged activation of microglia could contribute to the pathogenesis of neurodegenerative diseases. Furthermore, there is evidence that microglial toxins exert their neurotoxic effects via the NMDA receptor.[9,10]

Activated microglia display an increased expression of a range of molecules including complement receptor 3 (CR; also known as cluster-determinant (CD)11b) and major histocompatibility complex (MHC) class I and II.[11] One of the earliest molecules to be overexpressed is the 18 kDa translocator protein (TSPO) on the outer membrane of the mitochondria. The TSPO is widely distributed in the periphery but only minimally expressed in the healthy brain. It has been implicated in a range of biological functions including apoptosis, cell proliferation, immune system function, and steroidogenesis.[12] Imaging studies demonstrate a correlation between increased TSPO expression and central nervous system pathologies including Alzheimer's disease,[13] Huntington's disease,[14] and multiple sclerosis.[12] The TSPO is therefore considered as a relevant marker of neuroinflammation and neuronal injury and could be an attractive therapeutic target.

PK 11195 is the most studied TSPO ligand and has demonstrated anti-inflammatory and neuroprotective actions in vitro and in vivo.[7,15−17] Co-injection of PK 11195 and QUIN into the rat striatum has been shown to reduce microglial

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activation and increase neuronal survival.[7] However, PK 11195 is limited as a therapeutic agent due to inadequate pharmacokinetics, poor bioavailability and a high level of nonspecific binding.[18,19] Furthermore, PK 11195 has poor solubility properties. DPA-713 (**1**), DPA-714 (**2**), and propargyl-DPA (**3**) are novel pyrazolopyrimidine ligands which are selective for the TSPO, and which show more favorable characteristics in vivo. In addition, [^{11}C]DPA-713 and [^{18}F]DPA-714 are currently being utilized as a PET ligand for imaging neuroinflammation in animal and human studies.[20−23] These novel ligands display a higher signal-to-noise ratio, lower nonspecific binding, and lower lipophilicity. The neuroprotective characteristics of these ligands have not been investigated to date.

The aim of this study was to determine whether the novel TSPO ligands exerted neuroprotective effects in a QUIN rat model of excitotoxic injury.

RESULTS AND DISCUSSION

PK 11195 demonstrated significant neuroprotective effects in our rat model of excitotoxic injury, consistent with previous reports.[7] Furthermore, all three novel TSPO ligands DPA-713, DPA-714, and propargyl-DPA increased neuronal survival and inhibited microglial activation in the presence of QUIN. While previous evidence suggests that the neuroprotective effects exerted by PK 11195 are dose-dependent,[7] the dose-dependent effects of the novel TSPO ligands were not explored in this preliminary study. Rather, compounds were tested at a concentration of 5 nM, consistent with previous studies using PK 11195.[7]

Dimethyl sulfoxide (DMSO) also displayed some degree of neuroprotective effects, although through a pathway that is unlikely to involve microglia. This is consistent with previous studies demonstrating the broad spectrum of biological effects of DMSO: free radical scavenging,[24] anti-inflammatory effects,[25,26] and prevention glutamate-induced neuronal death in cell culture.[27] Furthermore, the neuroprotective properties of DMSO have already been demonstrated in a rat model of ischemia.[28]

The ligands and DMSO were first assayed for their ability to promote neuronal survival. We used NeuN immunohistochemistry to compare neuron loss of the QUIN-injected striatum with the unlesioned striatum in the same rat. While 10% DMSO and 40% DMSO had no significant effect on NeuN staining compared to 0.1 M PBS, striatal injection of QUIN caused a 65% reduction in NeuN immunoreactivity (Figure 1a), signifying significant neuronal loss. Interestingly, 10% DMSO and 40% DMSO significantly increased the NeuN-immunoreactive area in the striatum by 32% and 41%, respectively, indicative of a neuroprotective effect (Figure 1a). PK 11195, DPA-713, DPA-714, and propargyl-DPA exerted an additional neuroprotective effect by preventing neuronal cell death, compared to QUIN-injected controls in the presence of DMSO. DPA-714 and propargyl-DPA were the most effective, reducing neuronal loss by 38% (Figure 2). This was followed by PK 11195 then DPA-713 which reduced neuronal loss by 35% and 33%, respectively. There was no significant difference between the vehicle alone and PK 11195, DPA-714, or propargyl-DPA (Figure 2).

Excitotoxic injury is also denoted by microglial activation. Thus, the response of microglia to QUIN injection was also assessed 2 days postsurgery using OX-42 immunohistochemistry. Injection of QUIN caused a significant increase in OX-42

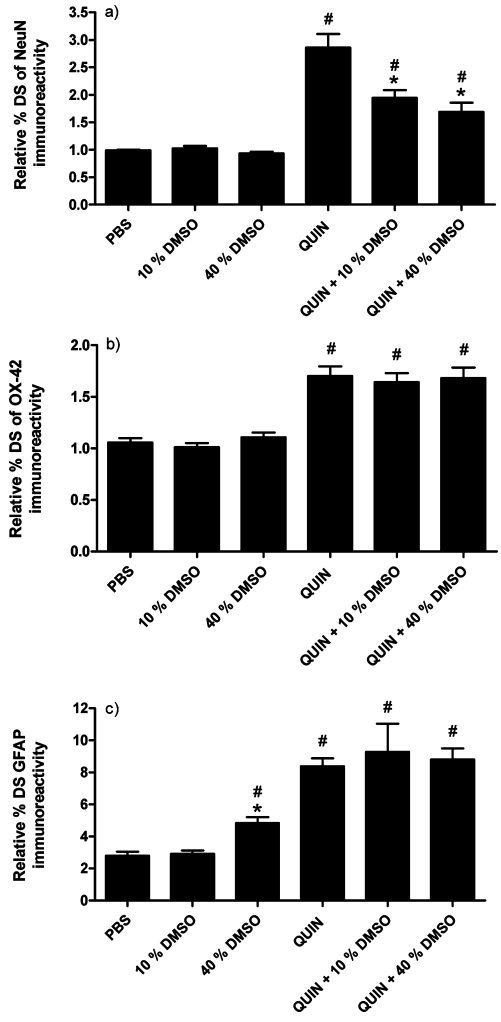

Figure 1. Effect of DMSO on the % DS score of NeuN (a), OX-42 (b), and GFAP (c) immunoreactivity in the striatum. % DS was calculated using the optical densities of the striatum and background area (corpus callosum). PBS, 40% DMSO, and QUIN (n = 8 animals per group); 10% DMSO, QUIN + 10% DMSO, and QUIN + 40% DMSO (n = 4 animals per group). Columns represent mean ± SEM. $^{*}p < 0.05$ compared to QUIN-injected rats. $^{\#}p < 0.05$ compared to PBS (Newman-Keuls multiple comparison test).

immunoreactivity (38% compared to 0.1 M PBS). Despite the neuroprotective effects displayed on neuronal survival, 10% DMSO and 40% DMSO had no effect on microglia in the presence of QUIN (Figure 1b), consistent with previous reports.[28] However, PK 11195, DPA-713, DPA-714, and propargyl-DPA inhibited microglial activation by 27%, 17%, 30%, and 37%, respectively (Figure 3), compared to QUIN and DMSO alone. There was no significant difference between the PK 11195, DPA-714, propargyl-DPA, and DMSO control groups (Figure 3).

Resting microglia usually display a ramified morphology with small cell bodies and thin processes in a normal brain. This was observed in the control group, with minimal numbers of small, ramified microglia (Figure 4). In response to injury, they rapidly change morphology to an amoeboid cell body with short, thick processes. This microglial response and change in morphology occurred following injection of QUIN into the striatum, with widespread presence of amoeboid microglia.

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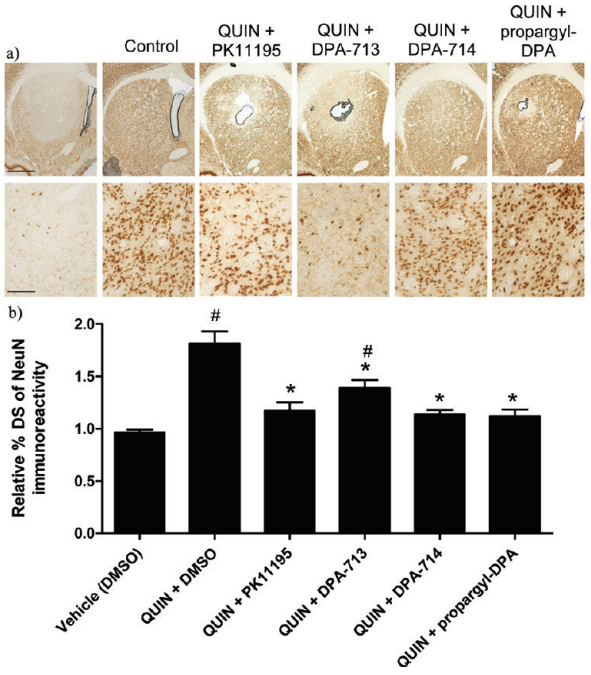

Figure 2. Effects of TSPO ligands on the % DS score of NeuN immunoreactivity in the striatum. % DS was calculated using the optical densities of the striatum and background area (corpus callosum). (a) NeuN staining in the striatum of control rats and QUIN lesioned rats coinjected with vehicle, PK 11195, DPA-713, DPA-714, or propargyl-DPA. High magnification photographs of NeuN immunostained striatal sections. Scale bars = 1 mm (upper panels), 27 μm (lower panels). (b) Columns represent mean ± SEM (n = 8 animals per group except vehicle n = 12 animals). *p < 0.05 compared to QUIN-injected rats. $^{\#}p$ < 0.05 compared to PBS (Newman-Keuls multiple comparison test).

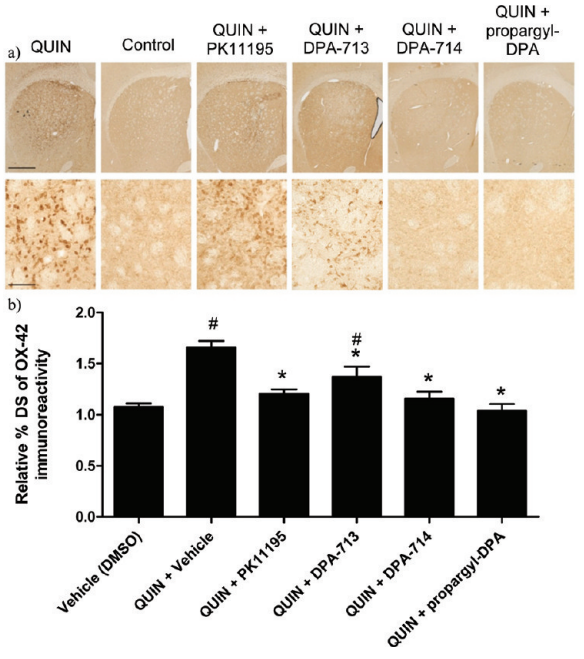

Figure 3. Effects of TSPO ligands on the % DS score of OX-42 immunoreactivity in the striatum. % DS was calculated using the optical densities of the striatum and background area (corpus callosum). (a) OX-42 staining in the striatum of control rats and QUIN lesioned rats coinjected with vehicle, PK 11195, DPA-713, DPA-714, or propargyl-DPA. High magnification photographs of OX-42 immunostained striatal sections. Scale bars = 1 mm (upper panels), 27 μm (lower panels). (b) Columns represent mean ± SEM (n = 8 animals per group except vehicle n = 12 animals). *p < 0.05 compared to QUIN-injected rats. $^{\#}p$ < 0.05 compared to PBS (Newman-Keuls multiple comparison test).

Amoeboid microglia were also observed to a lesser degree in the PK 11195 and DPA-713 treated groups (Figure 4).

Finally, considering the role of astrocytes in brain functions and communication between cells, we examined the effect of QUIN on astrocytosis. The astroglial response was evaluated 2 days postsurgery using GFAP immunohistochemistry. The injection of QUIN resulted in a significant increase (77%) in GFAP immunoreactivity compared to 0.1 M PBS, with immunoreactive astrocytes displaing enlarged cell bodies with thick processes compared to unlesioned animals (Figure 5). The injection of 10% DMSO with or without QUIN had no effect on GFAP immunoreactivity (Figure 1c). However, while injection of 40% DMSO with QUIN did not decrease astrogliosis, 40% DMSO alone significantly increased the astroglial response compared to 0.1 M PBS (42%; Figure 1c). There are very few studies investigating the effects of DMSO on astrocytes. One recent article reported increased astrogliosis following intracranial injection of 0.5 μL DMSO into the hippocampus,[29] only slightly higher than our 10% DMSO group at 0.4 μL. Therefore, it is possible that there is a threshold amount of DMSO required to increase astrogliosis. Conversely, different results may also be due to the injection of DMSO into different brain areas. Further investigation into the relationship between DMSO, astrocytes and neuroprotection is necessary.

As the two concentrations of DMSO had different effects on astrogliosis, we were unable to analyze the results of PK 11195 and the novel TSPO ligands together. We have instead presented their effects on astrocytes on separate graphs compared to their corresponding vehicle. None of the compounds tested significantly affected astrogliosis (Figure 5).

While the current study provides evidence for TSPO ligands with neuroprotective effects, the mechanisms underlying these effects still need to be investigated through future studies. TSPO ligands have been shown to exert their neuroprotective effects by inhibiting the production of reactive oxygen species and pro-inflammatory cytokines by microglia.[30] More specifically, the wide spectrum of anti-inflammatory effects of PK 11195, including inhibition of IL-1β, IL-6, TNF-α, and iNOS expression and reduction in lipid peroxidation and oxidative damage, have been demonstrated by Ryu and colleagues and indicate that inhibition of microglial activation confers neuroprotection against QUIN.[7] Similarly to PK 11195, we speculate that the novel compounds, DPA-713, DPA-714, and propargyl-DPA, would have similar anti-inflammatory effects.

On the other hand, the neuroprotective effects of DMSO seemed to be selective to increasing neuronal survival, independently of alterations to microglia or astrocytes. Thus, DMSO may exert neuroprotective effects by suppressing the NMDA and AMPA receptor response to glutamate in cell culture[27] or through its role as a free radical scavenger.[24] While it has been reported previously that DMSO is toxic to cells at a concentration greater than 2% in cell cultures of hippocampal neurons,[27] we did not observe any neurotoxic effects of DMSO at concentrations up to 40% injected directly into the striatum. This discrepancy is likely due to the use of in vitro versus in vivo modeling of excitotoxicity.

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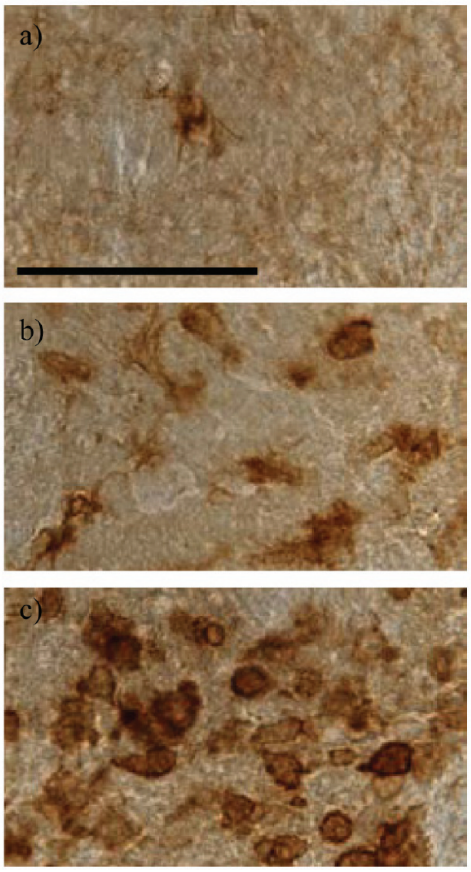

Figure 4. High magnification photographs of OX-42 immunostaining in the striatum. (a) Ramified microglia of the PBS group, (b) ramified and amoeboid microglia from one treatment group, (c) amoeboid microglia in the QUIN group. Scale bar = 100 μm.

In summary, we have demonstrated that DPA-713, DPA-714, and propargyl-DPA are all neuroprotective against QUIN injection into the rat striatum, by inhibiting microglial activation and promoting neuronal survival. Future studies should look at examining dose-related effects of these ligands, in order to compare their relative potencies. We also identified potent neuroprotective effects of DMSO, through an independent signaling pathway. These TSPO compounds have potential therapeutic applications as neuroprotective treatments for neurodegenerative diseases involving microglial activation such as Parkinson's disease, Alzheimer's disease and multiple sclerosis, and warrant further investigation.

METHODS

Chemistry. The TSPO ligands DPA-714 (**2**) and propargyl-DPA (**3**) were prepared from the phenol **4** using a standard *O*-alkylation procedure involving treatment with sodium hydride followed by the appropriate alkyl tosylate (Scheme 1). The phenol was derived from DPA-713 (**1**) following treatment with 48% aqueous hydrobromic acid in the presence of tetra-*n*-butylphosphonium bromide. DPA-713 (**1**) was prepared from commercially available methyl 4-methoxybenzoate via a 4-step procedure.[31]

Rat Surgery and Drug Administration. All experiments were carried out in accordance with French legislation and European Directives for the care of laboratory animals. A total of 65 male Sprague–Dawley rats, 8 weeks old at the beginning of the study (Centre d'élevage René Janvier, France), were used. Rats were housed in three per cage in a temperature (23 ± 0.5%) and humidity (43 ± 8%) controlled environment under 12/12 h light/dark cycle with

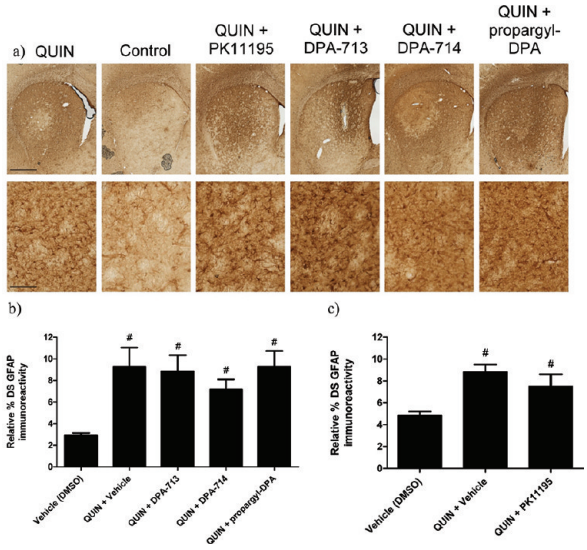

Figure 5. Effect of TSPO ligands on the % DS score of GFAP immunoreactivity in the striatum. % DS was calculated using the optical densities of the striatum and background area (corpus callosum). (a) High and low magnification photographs of GFAP immunostained striatal sections of control rats and QUIN lesioned rats coinjected with vehicle, PK 11195, DPA-713, DPA-714, or propargyl-DPA. Scale bars = 1 mm (upper panels), 27 μm (lower panels). (b,c) Columns represent mean ± SEM (n = 8 animals per group except vehicle and QUIN n = 4 animals). *p < 0.05 compared to QUIN-injected rats (Newman-Keuls multiple comparison test).

standard rat chow and water available ad libitum. Surgery was performed as previously described.[7] Briefly, rats (of weight 300–350 g) were deeply anesthetized using isoflurane gas (4% for induction, 2% for maintenance) and placed in a stereotactic frame (Lab Standard model 51600, Co., IL, USA). A burr hole was drilled and the animals were unilaterally injected with one of 10 different drug solutions (a total volume of 2 μL was injected over 5 min, at a flow rate of 0.4 μL/min) into the left striatum at the following coordinates: AP = +1.0 mm, ML = −3.0 mm, DV = −5.0 mm from bregma, according to the atlas of Paxinos and Watson.[32]

Drug Solutions. The TSPO ligands PK 11195, DPA-713, DPA-714, and propargyl-DPA were dissolved in DMSO to a stock concentration of 50 mM and then diluted with 0.1 M PBS immediately prior to injection (n = 8 animals per group). The amount of DMSO injected with each compound was either 10% (DPA-713, DPA-714 and propargyl-DPA) or 40% (PK 11195). Using a Hamilton syringe with a 26-gauge needle, 1 μL of of 5 nmol of drug solution was coinjected with 1 μL of 60 nmol QUIN (dissolved in 0.1 M PBS). Each group was tested against the vehicle (10% and 40% DMSO; n = 12 animals) and QUIN with the DMSO vehicle (n = 8).

Vehicle Evaluation. As the different compounds we injected required varying amounts of DMSO to dissolve them, we had two vehicle groups: 40% DMSO (n = 8 animals) and 10% DMSO (n = 4 animals). In the same model as described above, both vehicles were evaluated with QUIN (n = 4 animals per group) and were tested against 0.1 M PBS (n = 8 animals) and QUIN (n = 8 animals) to evaluate whether they possessed either neurotoxic or neuroprotective effects.

Tissue Preparation. Two days following surgery, the rats were sacrificed by deep anesthesia and perfused transcardially with 0.9% saline followed by 4% paraformaldehyde solution before decapitation. The brains were rapidly removed from the skull and postfixed overnight in fresh 4% paraformaldehyde solution. After postfixing, brains were cryoprotected in 30% sucrose solution for 48 h. Coronal sections (40 μm thick) were then cut in a cryomicrotome (Jung CM 3000, Leica) with five series of free-floating sections being collected for immunohistochemistry.

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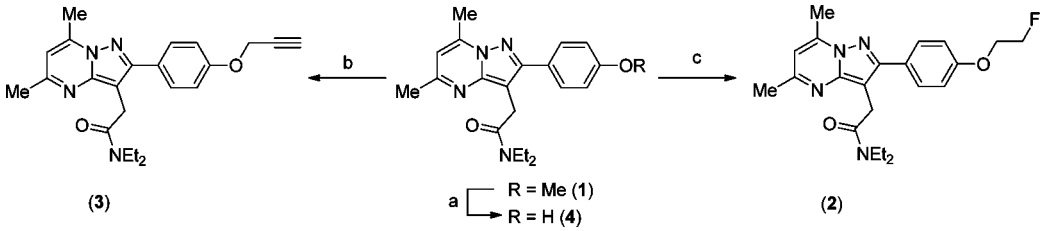
Scheme 1. Synthesis of DPA-713 (1), DPA-714 (2), and propargyl-DPA (3)[a]



[a]Reagents and conditions: (a) 48% v/v HBr, tetra-*n*-butylphosphonium bromide, 100 °C, 7 h, 54%; (b) NaH, DMF, 0 °C, then 2-fluoroethyl 4-methylbenzenesulfonate, 57%; (c) NaH, DMF, 0 °C, then prop-2-yn-1-yl 4-methylbenzenesulfonate, 77%.

Immunohistochemistry. The sections were incubated in 3% H_2O_2 and 50% ethanol for 20 min to quench endogenous peroxidases. Nonspecific binding of immunoreagents was then blocked by incubation in 20% normal horse serum (NHS) in 0.1 M PBS. Sections were incubated in either: (a) mouse anti-rat CD11B monoclonal antibody (CBL1512, Chemicon; diluted 1:1000), (b) mouse anti-neuronal nuclei (NeuN) monoclonal antibody (MAB377B, Chemicon; diluted 1:2000), or (c) rabbit anti-glial fibrillary acidic protein polyclonal antibody (Z0334, DakoCytomation; diluted 1:2000) for 48 h at 4 °C. All antibodies were diluted in 20% NHS/0.075% Triton X-100/PBS (PBH). Sections were then incubated in biotinylated antibody to either mouse or rabbit IgG (Amersham Biosciences; diluted 1:500 in PBH) for 2 h at room temperature. This was followed by incubation with Extravidin-Peroxidase (diluted 1:1000 in PBH; Sigma) for 2.5 h. Immunoreactive neurons were visualized with 3,3-diaminobenzamide (DAB; Sigma) for 10 min before 0.01% H_2O_2 was added to allow the reaction to run to completion. The DAB reaction was stopped by transferring sections to 0.1 M PBS. Washes in 0.1 M PBS were performed before each step excluding prior to incubation with the primary antibody and the DAB reaction. Sections were mounted free-floating onto gelatinized slides in 0.1 M PBS, air-dried, and then coverslipped with aqueous mounting medium (S3025, Dako).

Quantitative Analysis. Quantitative image analysis was performed using an Olympus BX51 microscope equipped with a 4× objective lens. Four immunostained sections (AP: +1.4, +1.2, +1.0 and +0.8 mm from bregma) were digitized into 650 × 515 pixels using a Leica DC 500 digital color camera under constant conditions of light, speed, and aperture. Images were analyzed using the image analysis program ImageJ (http://rsbweb.nih.gov/ij/). Optical densities (OD) of the striatum and the background area (a section of the unstained corpus callosum) of each rat were determined by assigning a numerical value between 0 (black) and 255 (white) to each pixel according to its gray scale value. For each hemisphere, the percent difference score (DS) of OD between these two areas was calculated using the following formula:[33,34]

$$DS(\%) = \left[(OD_{striatum} - OD_{background})/(OD_{striatum} + OD_{background})/2\right] \times 100$$

The mean percent DS obtained in the four striatal sections analyzed was calculated. The densitometric data presented represents the relative mean DS between the left and right (lesioned and unlesioned) hemispheres.

Statistical Analysis. Data were analyzed statistically using Statview software (version 5.0, Abacus Concepts Inc. Berkeley, CA, 1992). Statistical significance between the groups was assessed using one-way ANOVA and Neuman-Keuls multiple comparison posthoc test. All data are presented as the mean ± SEM, and significance was set at $p < 0.05$.

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Author Contributions

Katherine Leaver performed the rat lesions and drug dosing. Aaron Reynolds synthesized the drugs. Sylvie Bodard assisted with the animal work. Denis Guilloteau, Sylvie Chalon and Michael Kassiou conceived the experiments, analysis of data and final manuscript preparation.

Notes

The authors declare no competing financial interest.

REFERENCES

(1) Brouillet, E., Condé, F., Beal, M. F., and Hantraye, P. (1999) Replicating Huntington's disease phenotype in experimental animals. *Prog. Neurobiol. 59*, 427−468.

(2) Kreutzberg, G. W. (1996) Microglia: a sensor for pathological events in the CNS. *Trends Neurosci. 19*, 312−318.

(3) Leaver, K., Allbutt, H., Creber, N., Kassiou, M., and Henderson, J. (2008) Neuroprotective effects of a selective N-methyl-D-aspartate NR2B receptor antagonist in the 6-hydroxydopamine rat model of Parkinson's diesase. *Clin. Exp. Pharmacol. Physiol. 35*, 1388−1394.

(4) Loftis, J. M., and Janowsky, A. (2003) The N-methyl-D-aspartate receptor subunit NR2B: localization, functional properties, regulation, and clinical implications. *Pharmacol. Ther. 97*, 55−85.

(5) Schwarcz, R., Whetsell, W. O., and Mangano, R. M. (1983) Quinolinic acid: an endogenous metabolite that produces axon-sparing lesions in rat brain. *Science 219*, 316−318.

(6) Beal, M. F., Kowall, N. W., Ellison, D. W., Mazurek, M. F., Swartz, K. J., and Martin, J. B. (1986) Replication of the neurochemical characteristics of Huntington's disease by quinolinic acid. *Nature 321*, 168−171.

(7) Ryu, J. K., Choi, H. B., and McLarnon, J. G. (2005) Peripheral benzodiazepine receptor ligand PK11195 reduces microglial activation and neuronal death in quinolinic acid-injected rat striatum. *Neurobiol. Dis. 20*, 550−561.

(8) Tikka, T. M., and Koistinaho, J. E. (2001) Minocycline provides neuroprotection against N-methyl-D-aspartate neurotoxicity by inhibiting microglia. *J. Immunol. 166*, 7527−7533.

(9) Piani, D., Spranger, M., Frei, K., Schaffner, A., and Fontana, A. (1992) Macrophage-induced cytotoxicity of N-methyl-D-aspartate receptor positive neurons involves excitatory amino acids rather than reactive oxygen intermediates and cytokines. *Eur. J. Immunol. 22*, 2429−2436.

(10) Chao, C. C., Hu, S. X., Ehrlich, L., and Peterson, P. K. (1995) Interleukin-1 and tumor necrosis factor-[alpha] synergistically mediate neurotoxicity: Involvement of nitric oxide and of N-methyl-D-aspartate receptors. *Brain, Behav., Immun. 9*, 355−365.

(11) Tsuda, M., Inoue, K., and Salter, M. W. (2005) Neuropathic pain and spinal microglia: a big problem from molecules in 'small' glia. *Trends Neurosci. 28*, 101−107.

(12) Scarf, A. M., Ittner, L. M., and Kassiou, M. (2009) The translocator protein (18 kDa): Central nervous system disease and drug design. *J. Med. Chem. 52*, 581−592.

(13) Cagnin, A., Brooks, D. J., Kennedy, A. M., Gunn, R. N., Myers, R., Turkheimer, F. E., Jones, T., and Banati, R. B. (2001) *In vivo* measurement of activated microglia in dementia. *Lancet 358*, 461−467.

(14) Pavese, N., Gerhard, A., Tai, Y. F., Ho, A. K., Turkheimer, F., Barker, R. A., Brooks, D. J., and Piccini, P. (2006) Microglial activation correlates with severity in Huntington disease: A clinical and PET study. *Neurology 66*, 1638−1643.
(15) Klegeris, A., McGeer, E. G., and McGeer, P. L. (2000) Inhibitory action of 1-(2-chlorophenyl)-N-methyl-N-(1-methylpropyl)-3-isoquinolinecarboxamide (PK 11195) on some mononuclear phagocyte functions. *Biochem. Pharmacol. 59*, 1305−1314.
(16) Wilms, H., Claasen, J., Rohl, C., Sievers, J., Deuschl, G., and Lucius, R. (2003) Involvement of benzodiazepine receptors in neuroinflammatory and neurodegenerative diseases: evidence from activated microglial cells *in vitro*. *Neurobiol. Dis. 14*, 417−424.
(17) Belloli, S., Moresco, R. M., Matarrese, M., Biella, G., Sanvito, F., Simonelli, P., Turolla, E., Olivieri, S., Cappelli, A., Vomero, S., Galli-Kienle, M., and Fazio, F. (2004) Evaluation of three quinoline-carboxamide derivatives as potential radioligands for the *in vivo* PET imaging of neurodegeneration. *Neurochem. Int. 44*, 433−440.
(18) Dolle, F., Luus, C., Reynolds, A., and Kassiou, M. (2009) Radiolabelled molecules for imaging the translocator protein (18 kDa) using positron emission tomography. *Curr. Med. Chem. 16*, 2899−2923.
(19) Luus, C., Hanani, R., Reynolds, A., and Kassiou, M. (2010) The development of PET radioligands for imaging the translocator protein (18 kDa): What have we learned? *J. Labelled Compd. Radiopharm. 53*, 501−510.
(20) Doorduin, J., Klein, H., Dierckx, R., James, M., Kassiou, M., and de Vries, E. (2009) [^{11}C]-DPA-713 and [^{18}F]-DPA-714 as new PET tracers for TSPO: A comparison with [^{11}C]-(R)-PK11195 in a rat model of herpes encephalitis. *Mol. Imaging Biol. 11*, 386−398.
(21) Boutin, H., Chauveau, F., Thominiaux, C., Gregoire, M.-C., James, M. L., Trebossen, R., Hantraye, P., Dolle, F., Tavitian, B., and Kassiou, M. (2007) ^{11}C-DPA-713: A novel peripheral benzodiazepine receptor PET ligand for *in vivo* imaging of neuroinflammation. *J. Nucl. Med. 48*, 573−581.
(22) Chauveau, F., Van Camp, N., Dolle, F., Kuhnast, B., Hinnen, F. o., Damont, A., Boutin, H., James, M., Kassiou, M., and Tavitian, B. (2009) Comparative evaluation of the translocator protein radioligands ^{11}C-DPA-713, ^{18}F-DPA-714, and ^{11}C-PK11195 in a rat model of acute neuroinflammation. *J. Nucl. Med. 50*, 468−476.
(23) Endres, C. J., Pomper, M. G., James, M., Uzuner, O., Hammoud, D. A., Watkins, C. C., Reynolds, A., Hilton, J., Dannals, R. F., and Kassiou, M. (2009) Initial evaluation of ^{11}C-DPA-713, a novel TSPO PET ligand, in humans. *J. Nucl. Med. 50*, 1276−1282.
(24) Repine, J. E., Pfenninger, O. W., Talmage, D. W., Berger, E. M., and Pettijohn, D. E. (1981) Dimethyl sulfoxide prevents DNA nicking mediated by ionizing radiation or iron/hydrogen peroxide-generated hydroxyl radical. *Proc. Natl. Acad. Sci. U.S.A. 78*, 1001−1003.
(25) Wood, D. C., and Wood, J. (1975) Pharmacologic and biochemical considerations of dimethyl sulfoxide. *Ann. N.Y. Acad. Sci. 243*, 7−19.
(26) Phillis, J. W., Estevez, A. Y., and O'Regan, M. H. (1998) Protective effects of the free radical scavengers, dimethyl sulfoxide and ethanol, in cerebral ischemia in gerbils. *Neurosci. Lett. 244*, 109−111.
(27) Lu, C., and Mattson, M. P. (2001) Dimethyl sulfoxide suppresses NMDA- and AMPA-induced ion currents and calcium influx and protects against excitotoxic death in hippocampal neurons. *Exp. Neurol. 170*, 180−185.
(28) Farkas, E., Institóris, Á., Domoki, F., Mihály, A., Luiten, P. G. M., and Bari, F. (2004) Diazoxide and dimethyl sulphoxide prevent cerebral hypoperfusion-related learning dysfunction and brain damage after carotid artery occlusion. *Brain Res. 1008*, 252−260.
(29) Emamian, S., Naghdi, N., Sepehri, H., Jahanshahi, M., Sadeghi, Y., and Choopani, S. (2010) Learning impairment caused by intra-CA1 microinjection of testosterone increases the number of astrocytes. *Behav. Brain Res. 208*, 30−37.
(30) Zavala, F., Taupin, V., and Descamps-Latscha, B. (1990) *In vivo* treatment with benzodiazepines inhibits murine phagocyte oxidative metabolism and production of interleukin 1, tumor necrosis factor and interleukin-6. *J. Pharmacol. Exp. Ther. 255*, 442−450.
(31) Reynolds, A., Hanani, R., Hibbs, D., Damont, A., Pozzo, E. D., Selleri, S., Dollé, F., Martini, C., and Kassiou, M. (2010) Pyrazolo[1,5-a]pyrimidine acetamides: 4-Phenyl alkyl ether derivatives as potent ligands for the 18 kDa translocator protein (TSPO). *Bioorg. Med. Chem. Lett. 20*, 5799−5802.
(32) Paxinos, G., and Watson, C. (1998) *The rat brain in stereotaxic coordinates*, 4th ed., Academic Press, San Diego.
(33) Armitage, L. L., Mohapel, P., Jenkins, E. M., Hannesson, D. K., and Corcoran, M. E. (1998) Dissociation between mossy fiber sprouting and rapid kindling with low-frequency stimulation of the amygdala. *Brain Res. 781*, 37−44.
(34) Xu, H., Steven Richardson, J., and Li, X.-M. (2002) Dose-related effects of chronic antidepressants on neuroprotective proteins BDNF, Bcl-2 and Cu//Zn-SOD in rat hippocampus. *Neuropsychopharmacology 28*, 53−62.