Magnetic Resonance in Medicine 51:248–252 (2004)

MRI Detection of Early Endothelial Activation in Brain Inflammation

Nicola R. Sibson,[1] Andrew M. Blamire,[1] Martine Bernades-Silva,[2] Sophie Laurent,[3] Sébastien Boutry,[3] Robert N. Muller,[3] Peter Styles,[1] and Daniel C. Anthony[4]

MRI is an increasingly important clinical tool, but it is clear that conventional imaging fails to identify the full extent of lesion load in certain conditions, such as multiple sclerosis. The aim of this study was to determine whether a novel contrast agent (Gd-DTPA-B(sLe^X)A, which contains an sLe^X mimetic moiety that enables it to bind to the adhesion molecule E-selectin) can be used to identify endothelial activation in the brain. Microinjection of the proinflammatory cytokines IL-1β or TNF-α into the striatum of Wistar rats rapidly induces focal adhesion molecule expression on the endothelium in the absence of MRI-visible changes. This phenomenon was used to investigate the potential of Gd-DTPA-B(sLe^X)A to reveal MRI-invisible brain pathology. T_1-weighted serial images were acquired in anesthetized animals before and after administration of Gd-DTPA-B(sLe^X)A, 3–4 hr after cytokine was injected intracerebrally. Both TNF-α and IL-1β up-regulated E-selectin on the brain endothelium, which correlated with increased signal intensity observed after administration of the novel contrast agent. No enhancement was visible with the nonselective contrast agent Gd-DTPA-BMA, indicating that there was no leakage of the agent across the blood–brain barrier (BBB) or nonselective binding to the endothelium. These data demonstrate the potential of such contrast agents for the early detection of brain injury and inflammation. Magn Reson Med 51:248–252, 2004.

Key words: magnetic resonance; brain; inflammation; E-selectin; rat

Multiple sclerosis (MS) is the archetypal inflammatory demyelinating disease of the central nervous system (CNS). The pathology of this disease is characterized by loss of myelin from regions of CNS white matter, and loss of axons (1). Evidence from both neuropathological (2) and MR (3) investigations has shown that axon loss may occur early in the disease process. In an experimental model that displays many of the histopathological hallmarks of MS, we have found that axon injury is not restricted to any particular phase of the inflammatory response. In particular axon injury is not restricted to the period of blood–brain barrier (BBB) breakdown, as detected by gadolinium enhancement in MRI or other conventional imaging techniques (4). Novel contrast agents linked to appropriate biomarkers will enable the visualization of lesions within the brain that cannot be seen with conventional techniques, and will be of use in a broad range of CNS pathologies.

It is clear that solute and cellular permeabilities are not equivalent with regard to the BBB, and that leukocyte populations are able to cross an intact BBB (5–7). However, in acute inflammatory lesions the brain endothelium becomes activated to facilitate leukocyte recruitment (8). An essential requirement for leukocyte recruitment from the circulation is the expression of adhesion molecules on the endothelium (9), which is one of the earliest events in an inflammatory response. The arrest of freely flowing leukocytes, and their subsequent rolling along the endothelium are thought to be mediated by the selectins, which bind to carbohydrate ligands (10,11). Both E-selectin and P-selectin are expressed on activated endothelium, but not on resting brain endothelium, and P-selectin is also present on platelets. Both of these selectins interact with the Sialyl Lewis^X (sLe^X) carbohydrate associated with CD15 on the surface of neutrophils. Their function is to slow the circulating leukocytes as they roll along the endothelium. The stereotaxic intracerebral microinjection of IL-1β has been shown to induce focal expression of E- and P-selectin in the injected hemisphere in the absence of BBB breakdown (12). The microinjection of IL-1β also gives rise to delayed selectin-dependent recruitment of neutrophils across the intact BBB. In contrast, microinjection of TNF-α into the brain gives rise to a pattern of leukocyte recruitment to the brain that is quite distinct from the action of IL-1β. TNF-α induces delayed monocyte and T-cell recruitment to the brain across an intact BBB (13,14), but it is unclear whether the TNF-α-dependent recruitment of monocytes and T-cells is also associated with the expression of E- and P-selectin.

At present, gadolinium (Gd)-DTPA compounds are among the most widely used paramagnetic contrast agents in routine MRI examinations. The safety of these compounds is based on the very strong chelating capability of DTPA. However, these agents are currently limited, by their lack of specificity, to the detection of BBB permeability in the CNS as a consequence of leakage into and accumulation within the brain parenchyma. We recently described the synthesis of a novel contrast agent, based on Gd-DTPA, to target sites of inflammation in which E-selectin is expressed (15). The structure of a mimetic of sLe^X, namely 3-([2-(-D-mannopyranosyloxy)phenyl])phenylacetic acid, was coupled to DTPA via a flexible alkyl spacer and the amide linkage to produce a compound known as Gd-DTPA-

[1]MRC Biochemical and Clinical Magnetic Resonance Unit, Department of Biochemistry, University of Oxford, Oxford, United Kingdom.

[2]CNS Inflammation Group, School of Biological Sciences, University of Southampton, Southampton, United Kingdom.

[3]NMR Laboratory, Department of Organic Chemistry, University of Mons-Hainaut, Mons, Belgium.

[4]Molecular Neuropathology Laboratory, School of Biological Sciences, University of Southampton, Southampton, United Kingdom.

Grant sponsor: Medical Research Council; Grant sponsor: ARC, French Community of Belgium; Grant number: 00/05-258.

*Correspondence to: Dr. Nicola R. Sibson, MRC Biochemical and Clinical Magnetic Resonance Unit, Dept. of Biochemistry, University of Oxford, South Parks Rd., Oxford, OX1 3QU, UK. E-mail: nicola.sibson@bioch.ox.ac.uk

Received 8 August 2003; revised 1 October 2003; accepted 3 October 2003.
DOI 10.1002/mrm.10723
Published online in Wiley InterScience (www.interscience.wiley.com).

B(sLe^x)A. The aim of the current study was to determine whether this novel contrast agent could be used to identify endothelial activation in the brain. Using focal intracerebral microinjections of IL-1β and TNF-α to induce selectin expression, we found that Gd-DTPA-B(sLe^x)A can reveal the presence of inflammation in the brain that is otherwise invisible by MRI.

MATERIALS AND METHODS

Animal Preparation

Adult male Wistar rats (232 ± 55 g; Harlan-Olac, UK) were anesthetized with 2.5% isoflurane in 70% N_2O:30% O_2. By means of a <50 μm-tipped glass pipette, 1 μl of cytokine solution (NIBSC, Potters Bar, UK) was injected stereotaxically over a 10-min period, 1 mm anterior and 3 mm lateral to the bregma, at a depth of 4 mm into the left striatum. The animals were injected with either 1 ng/μl of recombinant rat IL-1β (N = 6) or 1.5 μg/μl of recombinant rat TNF-α (N = 6), each in 0.1% BSA in low-endotoxin saline. A cannula was inserted into a tail vein for the administration of contrast agent during the MRI experiment. The rats were positioned in the MRI probe (3.4 cm i.d.; Alderman-Grant resonator) using an in-house-built bite-bar with nose restraint. During MRI, anesthesia was maintained with 1.0–1.5% isoflurane in 70% N_2O:30% O_2, ECG was monitored via subcutaneous electrodes, and body temperature was maintained at ~37°C by a circulating warm-water system. All procedures were approved by the United Kingdom Home Office.

MRI

MR images were acquired using a 7T horizontal bore magnet with a Varian Inova spectrometer (Varian, Palo Alto, CA). Anatomical scout images in both the horizontal and coronal planes were acquired using a fast spin-echo T_2-weighted sequence, with a repetition time (TR) of 3 s, echo time (TE) of 80 ms, matrix size 128 × 126, and field of view (FOV) of 3 cm × 3 cm. Slice thickness was 2 mm in the horizontal plane and 1 mm in the coronal plane. These scout images enabled us to visualize the injection site as a small hyperintense region, as observed previously (16,17), through which the target slice was positioned. Spin-echo T_1-weighted images (TR = 500 ms, TE = 20 ms) were acquired in the horizontal plane, with a slice thickness of 2 mm, a matrix size of 128 × 256, and a FOV of 3 cm × 6 cm.

Experimental Protocol

The rats were divided into three groups (A–C), and studies were performed to investigate the expression of the adhesion molecule E-selectin following cytokine injection into the striatum.

Group A

The animals (N = 3) were placed in the magnet 2 hr after IL-1β was injected intracerebrally, and scout images were acquired to position the target scan. A pre-contrast-agent, T_1-weighted image was acquired, and at 2 hr 45 min after IL-1β injection, 0.8 ml Gd-DTPA-B(sLe^x)A (27.8 mM solution) was injected intravenously over a period of 2–3 min via the tail vein cannula. T_1-weighted images were subsequently acquired every 10 min, beginning immediately post-contrast injection, for 1 hr. This time period was chosen based on previous observations of E-selectin expression 3–4 hr post IL-1β injection (12).

Group B

The rats (N = 3) were treated as in Group A, except that an equivalent dose (0.1 mmol/kg) of the conventional intravascular contrast agent Gd-DTPA-BMA (Omniscan, Nycomed Amersham, UK) was injected in an 0.8-ml volume instead of the Gd-DTPA-B(sLe^x)A. Again, T_1-weighted images were acquired every 10 min, beginning immediately post-contrast injection, for 1 hr.

Group C

The animals (N = 3) were placed in the magnet 3 hr after TNF-α was injected intracerebrally, and scout images were acquired to position the target scan. A pre-contrast-agent, T_1-weighted image was acquired, and 4 hr after TNF-α injection, 0.8 ml Gd-DTPA-B(sLe^x)A (27.8 mM solution) was injected intravenously over a period of 2–3 min via the tail vein cannula. T_1-weighted images were subsequently acquired every 10 min, beginning immediately post-contrast injection, for 1 hr. This time period was chosen based on our previous immunohistochemical and MRI observations that the earliest macrophage recruitment to the CNS occurs at ~4 hr post-TNF-α injection, and there is no BBB breakdown within the brain parenchyma earlier than 24 hr post-TNF-α injection (16). Three additional animals were injected with TNF-α in the same way, but did not undergo the MRI protocol. These animals were killed for immunohistochemistry (described below) 4 hr after TNF-α injection.

Histological Analysis

Following MRI, the animals injected with IL-1β and Gd-DTPA-B(sLe^x)A, and all animals injected with TNF-α were deeply anesthetized with sodium pentobarbitone, and transcardially perfused with 0.9% heparinized saline. Their brains were rapidly removed and immediately frozen in Tissue-Tek OCT embedding compound (Miles Inc., Elkhart, USA). Frozen 10-μm-thick coronal serial sections of fresh-frozen tissue were cut on a cryostat and mounted on 3-aminopropyltriethoxysilane-coated slides. The sections were air-dried and fixed in ice-cold absolute ethanol for 10 min prior to staining. The primary antibodies (anti-E-selectin monoclonal antibody RME-1 and anti-P-selectin monoclonal antibody RP-2) were incubated on separate sections for 2 hr. They were detected with the use of biotinylated secondary antibodies and an ABC Elite detection kit (Vector Laboratories, Peterborough, UK), with 3,3′-diaminobenzidine (DAB) as the substrate. Sections used for counting blood vessels were not counterstained. Sections used for photographic purposes were counterstained with hematoxylin. For each animal, five fields were chosen randomly in the striatum in the injected (E-selectin and laminin) and uninjected (laminin) hemisphere, and the

FIG. 1. The expression of E-selectin (brown staining) on the cerebral endothelium in the hemisphere injected with (a) IL-1β or (b) TNF-α in adult animals. Cresyl violet was used as a counterstain.

positively-stained vessels were counted with the use of Leica Qwin image analysis software (Leica, UK). All vessels, whether longitudinal, transverse, or branched, were each counted as one single blood vessel. The number of E-selectin positive vessels per square millimeter were expressed as a percentage of the laminin positive vessels to account for variations among the animals.

RESULTS

E-Selectin Expression

To examine the potential of the novel contrast agent targeted at inflammation, we microinjected IL-1β and TNF-α into the brain parenchyma to generate focal inflammatory lesions in the absence of BBB breakdown. In agreement with our previous findings (12), intracerebral injection of IL-1β induced the up-regulation of E- and P-selectin. At 4 hr, before any appreciable neutrophil recruitment occurred, 76% of the vessels in the injected hemisphere were E-selectin-positive (Fig. 1a). E-selectin expression was also observed on vessels in the meninges and in the choroid plexus. P-selectin expression was always less prominent than E-selectin (at most, only 15% of the vessels appeared positive). Like E-selectin, P-selectin immunoreactivity was also detected on vessels in the meninges and in the choroid plexus. Following an intracerebral injection of TNF-α, all animals exhibited expression of E-selectin within the injected striatum (Fig. 1b), although the proportion of E-selectin positive vessels was lower than the IL-1β-injected animals (25% vessels were positive at 4 hr). P-selectin was up-regulated to a similar extent in the TNF-α-injected animals compared to those injected with IL-1β.

Interleukin-1β Injections

To determine whether there would be any change in signal intensity over the 50-min imaging period with a conventional contrast agent, we injected the nonselective contrast agent Gd-DTPA-BMA. Following the intravenous injection of Gd-DTPA-BMA, there was no evidence of any increase in signal intensity at any time point that might have been indicative of low-grade breakdown of the BBB, or evidence that this nonselective contrast agent could bind to the activated brain endothelium (Fig. 2a).

In the animals injected with IL-1β and the contrast agent Gd-DTPA-B(sLe^X)A, a region of increased signal intensity was seen in the injected striatum (left), and the maximal signal change apparently occurred ~50 min after contrast agent injection (Fig. 2b). This finding was consistent in all of the three animals injected with IL-1β and Gd-DTPA-B(sLe^X)A. The signal enhancement was often patchy in appearance, in accord with the heterogeneous nature of the cerebral microvasculature. In addition, the areas of contrast enhancement correlated spatially with those areas exhibiting E-selectin up-regulation, as described immunocytochemically.

TNF-α Injections

In one of three animals injected with TNF-α and Gd-DTPA-B(sLe^X)A, a region of increased signal intensity was seen in the injected striatum, which was most obvious in the difference image (50 min-pre). Again, the signal change appeared to be maximal ~50 min after contrast agent injection (Fig. 2c). As for IL-1β, in the animal that did exhibit contrast enhancement, this region correlated spatially with those areas exhibiting E-selectin upregulation in the animals that were studied immunocytochemically.

DISCUSSION

In this study we have demonstrated that the cytokines IL-1β and TNF-α induce the expression of selectins on the brain microvasculature, and that this can be imaged in vivo with the novel contrast agent Gd-DTPA-B(sLe^X)A. Moreover, at the time points when signal enhancement was observed with the Gd-DTPA-B(sLe^X)A, no abnormalities are detectable using either non-enhanced imaging methods (T_1-, T_2-, or diffusion-weighted) or conventional Gd-DTPA-BMA contrast enhancement (16,17). Thus, the use of this novel contrast agent reveals pathology that is otherwise invisible by MRI. Though the signal enhancement was moderate, this study demonstrates that compounds conjugated to ligands of adhesion molecules are likely to be useful in clinical medicine.

Signal enhancement with Gd-DTPA-B(sLe^X)A was reliably detected in animals injected with IL-1β, but less so in those animals injected with TNF-α. This probably reflects the lower expression of E-selectin following TNF-α injection (25% of vessels) compared to IL-1β injection (75% of vessels). The dose of Gd-DTPA-B(sLe^X)A used was similar to the dose of Gd-DTPA-BMA that is routinely used in our

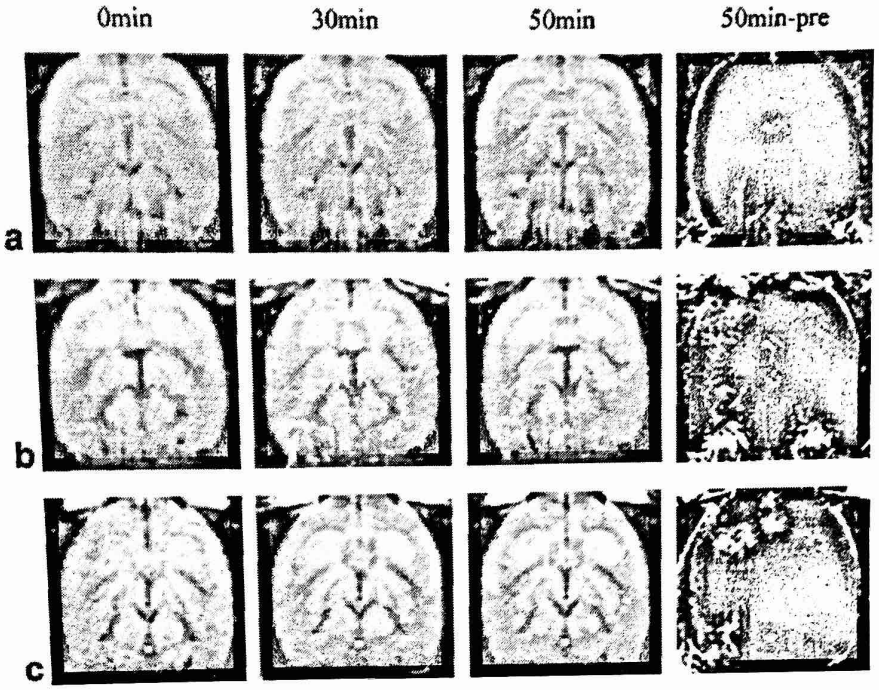

FIG. 2. **a:** T_1-weighted images from an animal injected intracerebrally with IL-1β and intravenously with Gd-DTPA-BMA, acquired 0, 30, and 50 min after contrast agent administration. No changes in signal intensity were observed at any time (as shown by the difference (50 min-pre) image), indicating that there is no BBB breakdown and entry of the contrast agent into the brain tissue. **b:** T_1-weighted images from an animal injected intracerebrally with IL-1β and intravenously with Gd-DTPA-B(sLe^X)A acquired at 0, 30, and 50 min after contrast agent administration. A region of increased signal intensity can be seen in the injected striatum (left). The degree of signal change appeared to be maximal ~50 min after contrast agent injection, and is more apparent in the difference (50 min-pre) image. This finding was consistent in the three animals injected with IL-1β and Gd-DTPA-B(sLe^X)A. **c:** T_1-weighted images from an animal injected intracerebrally with TNF-α and intravenously with Gd-DTPA-B(sLe^X)A, acquired 0, 30, and 50 min after contrast agent administration. A region of increased signal intensity can be seen in the injected striatum (left), and again the degree of signal change appeared to be maximal at ~50 min. This enhancement was more obvious in the difference image (50 min-pre), but was only observed in a third of the animals.

studies to investigate BBB breakdown. Restrictions on the volume of compound that we are allowed to administer prevented the use of an increased load of Gd-DTPA-B(sLe^X)A. It is also possible, therefore, that the level of E-selectin expression induced by TNF-α is close to the threshold detection level for Gd-DTPA-B(sLe^X)A at the dose used. The expression of E- and P-selectins on cerebral blood vessels has been described previously in models of permanent or transient middle cerebral artery occlusion in rats and nonhuman primates (18–21), and after the microinjection of IL-1β (12). In addition, both IL-1β and TNF-α have been shown to be capable of inducing the expression of E- and P-selectin on brain-derived microvascular endothelial cells in vitro (22). However, this is the first account showing that selectin expression can be induced on the brain microvasculature by TNF-α in vivo.

The lack of enhancement in the animals injected with IL-1β and Gd-DTPA-BMA demonstrated that the signal enhancement observed with IL-1β and Gd-DTPA-B(sLe^X)A was not due to breakdown of the BBB and entry of the contrast agent into the brain tissue. Previous MRI studies of TNF-α-induced CNS inflammation have demonstrated that there is no BBB breakdown at time points earlier than 24 hr (16). The conventional contrast agent used in this study was Gd-DTPA-BMA (Omniscan), which is a bis(amide) complex. Since the sLe^X mimetic is also a bis(amide) complex, Gd-DTPA-BMA is a more appropriate reference agent for this study than the other conventionally-used contrast agent Gd-DTPA (Magnevist), which is a pentacarboxylate. The bis(amides) are neutral compounds, while the Magnevist has a global 2– charge that is compensated for by counter ions (usually two molecules of glucamide). The relaxivity of the Gd-DTPA-B(sLe^X)A is 3.9 s^{-1} mM^{-1} at 0.5 T, and 3.5 s^{-1} mM^{-1} at 1.5T, which is identical to that of both Gd-DTPA and Gd-DTPA-BMA.

The targeting of contrast agents to specific molecules involved in pathological processes has been commonplace in positron emission tomography (PET) studies for some years (23–28); however, this approach has only recently been applied to MRI contrast agents. To date, only two studies of such agents have been reported (in addition to our work describing the synthesis and preliminary experiments with the agent described here (15)), and those studies were either ex vivo (29) or in vitro (30). Thus, the current study is the first in which the detection of contrast enhancement by such an agent in the brain in vivo has been reported. In the case of the ex vivo study (29), antibody-conjugated paramagnetic liposomes were targeted to intercellular adhesion molecule-1 (ICAM-1), and used to detect up-regulation of this endothelial leukocyte receptor

in a mouse model of experimental autoimmune encephalitis. Contrast enhancement was detected on T_1-weighted images at 9.4T with an average scan time of 7 hr. In the current study, contrast enhancement was detected in vivo at 7T with a scan time of 4 min. In the in vitro study (30), covalent conjugates of cross-linked iron oxide nanoparticles and high-affinity antihuman E-selectin antibody fragment were used to image E-selectin expression in human endothelial cell culture on exposure to IL-1β. Loss of signal was observed in cells treated with IL-1β and incubated with the anti-E-selectin contrast agent. Given the greater relaxivities of the iron oxide nanoparticles compared with gadolinium per particle/molecule, it is likely that vectors linked to those superparamagnetic crystals may prove more sensitive than the agent used in the current study.

Although the degree of signal enhancement was modest in this preliminary study, the current data demonstrate the potential of such contrast agents for the early detection of brain injury and inflammation. The targeting of contrast agents to distinct molecules associated with tissue pathology provides the potential to investigate disease noninvasively in vivo at the molecular level. Endothelial adhesion molecules are an important target for contrast-enhanced MRI because their expression is among the earliest events in the inflammatory response. Consequently, this approach may provide early indications of neurological disease and injury at times when conventional MRI methods are ineffective in identifying these processes but administration of therapy may be optimal.

ACKNOWLEDGMENTS

The authors thank Dr. J.-M. Colet for his contribution in the early stages of this project.

REFERENCES

1. Ferguson B, Matyszak MK, Esiri MM, Perry VH. Axonal damage in acute multiple sclerosis lesions. Brain 1997;120(Pt 3):393–399.
2. Ganter P, Prince C, Esiri MM. Spinal cord axonal loss in multiple sclerosis: a post-mortem study. Neuropathol Appl Neurobiol 1999;25: 459–467.
3. Arnold DL, Matthews PM, Francis G, Antel J. Proton magnetic resonance spectroscopy of human brain in vivo in the evaluation of multiple sclerosis: assessment of the load of disease. Magn Reson Med 1990;14:154–159.
4. Newman TA, Woolley ST, Hughes PM, Sibson NR, Anthony DC, Perry VH. T-cell- and macrophage-mediated axon damage in the absence of a CNS-specific immune response: involvement of metalloproteinases. Brain 2001;124:2203–2214.
5. Hickey WF, Hsu BL, Kimura H. T-lymphocyte entry into the central nervous system. J Neurosci Res 1991;28:254–260.
6. Hickey WF, Vass K, Lassmann H. Bone marrow-derived elements in the central nervous system: an immunohistochemical and ultrastructural survey of rat chimeras. J Neuropathol Exp Neurol 1992;51:246–256.
7. Perry VH, Bell MD, Brown HC, Matyszak MK. Inflammation in the nervous system. Curr Opin Neurobiol 1995;5:636–641.
8. Bolton SJ, Anthony DC, Perry VH. Loss of the tight junction proteins occludin and zonula occludens-1 from cerebral vascular endothelium during neutrophil-induced blood-brain barrier breakdown in vivo. Neuroscience 1998;86:1245–1257.
9. Winn R, Vedder N, Ramamoorthy C, Sharar S, Harlan J. Endothelial and leukocyte adhesion molecules in inflammation and disease. Blood Coagul Fibrinolysis 1998;9(Suppl 2):S17–23.
10. Ebnet K, Vestweber D. Molecular mechanisms that control leukocyte extravasation: the selectins and the chemokines. Histochem Cell Biol 1999;112:1–23.
11. Vestweber D, Blanks JE. Mechanisms that regulate the function of the selectins and their ligands. Physiol Rev 1999;79:181–213.
12. Bernardes-Silva M, Anthony DC, Issekutz AC, Perry VH. Recruitment of neutrophils across the blood-brain barrier: the role of E- and P-selectins. J Cereb Blood Flow Metab 2001;21:1115–1124.
13. Glabinski A, Krajewski S, Rafalowska J. Tumor necrosis factor-alpha induced pathology in the rat brain: characterization of stereotaxic injection model. Folia Neuropathol 1998;36:52–62.
14. Anthony DC, Bolton SJ, Fearn S, Perry VH. Age-related effects of interleukin-1b on polymorphonuclear neutrophil-dependent increases in blood-brain barrier permeability in rats. Brain 1997;120:435–444.
15. Fu Y, Laurent S, Muller RN. Synthesis of a Sialyl Lewis^x mimetic conjugated with DTPA, a potential ligand of new contrast agents for medical imaging. Eur J Org Chem 2002;2002:3966–3973.
16. Sibson N, Blamire A, Gauldie J, Perry V, Styles P, Anthony D. TNF-α reduces cerebral blood volume and disrupts tissue homeostasis via an endothelin and TNFR2-dependent pathway. Brain 2002;125:2446–2459.
17. Blamire AM, Anthony DC, Rajagopalan B, Sibson NR, Perry VH, Styles P. Interleukin-1beta-induced changes in blood-brain barrier permeability, apparent diffusion coefficient, and cerebral blood volume in the rat brain: a magnetic resonance study. J Neurosci 2000;20:8153–8159.
18. Zhang RL, Chopp M, Zhang ZG, Phillips ML, Rosenbloom CL, Cruz R, Manning A. E-selectin in focal cerebral ischemia and reperfusion in the rat. J Cereb Blood Flow Metab 1996;16:1126–1136.
19. Zhang R, Chopp M, Zhang Z, Jiang N, Powers C. The expression of P- and E-selectins in three models of middle cerebral artery occlusion. Brain Res 1998;785:207–214.
20. Okada Y, Copeland BR, Mori E, Tung MM, Thomas WS, del Zoppo GJ. P-selectin and intercellular adhesion molecule-1 expression after focal brain ischemia and reperfusion. Stroke 1994;25:202–211.
21. Haring HP, Berg EL, Tsurushita N, Tagaya M, del Zoppo GJ. E-selectin appears in nonischemic tissue during experimental focal cerebral ischemia. Stroke 1996;27:1386–1391.
22. Barkalow FJ, Goodman MJ, Gerritsen ME, Mayadas TN. Brain endothelium lack one of two pathways of P-selectin-mediated neutrophil adhesion. Blood 1996;88:4585–4593.
23. Cagnin A, Myers R, Gunn RN, Lawrence AD, Stevens T, Kreutzberg GW, Jones T, Banati RB. In vivo visualization of activated glia by [11c](r)-pk11195 PET following herpes encephalitis reveals projected neuronal damage beyond the primary focal lesion. Brain 2001;124: 2014–2027.
24. Cagnin A, Brooks DJ, Kennedy AM, Gunn RN, Myers R, Turkheimer FE, Jones T, Banati RB. In-vivo measurement of activated microglia in dementia. Lancet 2001;358:461–467.
25. Pappata S, Levasseur M, Gunn RN, Myers R, Crouzel C, Syrota A, Jones T, Kreutzberg GW, Banati RB. Thalamic microglial activation in ischemic stroke detected in vivo by PET and [11c]pk1195. Neurology 2000; 55:1052–1054.
26. Ramsay SC, Weiller C, Myers R, Cremer JE, Luthra SK, Lammertsma AA, Frackowiak RS. Monitoring by PET of macrophage accumulation in brain after ischaemic stroke. Lancet 1992;339:1054–1055.
27. Banati RB, Goerres GW, Myers R, Gunn RN, Turkheimer FE, Kreutzberg GW, Brooks DJ, Jones T, Duncan JS. [11c](r)-pk11195 positron emission tomography imaging of activated microglia in vivo in Rasmussen's encephalitis. Neurology 1999;53:2199–2203.
28. Banati RB, Newcombe J, Gunn RN, Cagnin A, Turkheimer F, Heppner F, Price G, Wegner F, Giovannoni G, Miller DH, Perkin GD, Smith T, Hewson AK, Bydder G, Kreutzberg GW, Jones T, Cuzner ML, Myers R. The peripheral benzodiazepine binding site in the brain in multiple sclerosis: quantitative in vivo imaging of microglia as a measure of disease activity. Brain 2000;123(Pt 11):2321–2337.
29. Sipkins DA, Gijbels K, Tropper FD, Bednarski M, Li KC, Steinman L. Icam-1 expression in autoimmune encephalitis visualized using magnetic resonance imaging. J Neuroimmunol 2000;104:1–9.
30. Kang HW, Josephson L, Petrovsky A, Weissleder R, Bogdanov Jr A. Magnetic resonance imaging of inducible E-selectin expression in human endothelial cell culture. Bioconjug Chem 2002;13:122–127.