ELSEVIER

Journal of Neuroscience Methods 144 (2005) 183–191

JOURNAL OF NEUROSCIENCE METHODS

www.elsevier.com/locate/jneumeth

Characterization of a rat model to study acute neuroinflammation on histopathological, biochemical and functional outcomes

Andrea Ambrosini, Gaëlle Louin, Nicole Croci, Michel Plotkine, Mehrnaz Jafarian-Tehrani*

Laboratoire de Pharmacologie (UPRES EA 2510), Université René Descartes, 4, avenue de l'Observatoire, 75270 Paris cedex 06, France

Received 16 August 2004; received in revised form 1 November 2004; accepted 4 November 2004

Abstract

Neuroinflammation is one of the events occurring after acute brain injuries. The aim of the present report was to characterize a rat model to study acute neuroinflammation on the histopathological, biochemical and functional outcomes. Lipopolysaccharide (LPS), known as a strong immunostimulant, was directly injected into the hippocampus. The spatiotemporal evolution of inducible NOS (iNOS) and cell death was studied from 6 h to 7 days. A perfect time course correlation was observed between iNOS immunoreactivity and iNOS activity showing an acute, expansive and transient iNOS induction in the hippocampus with a peak at 24 h. It was associated with a marked increase in NO metabolite (NO_x) levels, and a high level of myeloperoxidase (MPO) activity. This inflammation precedes a massive cellular loss including at least neurons and astrocytes, and a drop of constitutive NOS activity, restrictive to the ipsilateral hippocampus from 48 h after LPS injection. Moreover, sensorimotor function impairment occurred from 24 h to 7 days with a maximum at 24 h post-LPS injection. Therefore, we characterized an in vivo model of acute neuroinflammation and neurodegeneration, in relation with a neurological deficit, which may be a powerful tool for mechanistic studies and for further evaluation of the potential neuroprotective agents.

Keywords: Brain injury; Lipopolysaccharides; Nitric oxide; Nitric oxide synthases; Myeloperoxidase activity; NeuN; GFAP; Neurological score

1. Introduction

Acute brain injuries such as ischemia and trauma have in common some similar pathophysiological mechanisms leading to brain tissue destruction and functional impairment. Neuroinflammation is one of those, affecting the outcome of brain injuries. While acute inflammatory response may participate in secondary brain lesions, more delayed response may be on the contrary beneficial and reparative (Bramlett and Dietrich, 2004; Leker and Shohami, 2002). Thus the area of acute neuroinflammation, taken as a potential target for neuroprotective strategies, is under active investigation.

One of the mediators produced following brain inflammation is nitric oxide (NO) and is believed to play a dual role, depending on the type of NO synthase (NOS) isoform producing NO, its level and the cellular environment where it is produced (Zamora et al., 2000). NO is produced from L-arginine either by constitutive Ca^{2+}-dependent NOS (cNOS), including neuronal NOS (nNOS) and endothelial NOS (eNOS), or inducible Ca^{2+}-independent NOS (iNOS) (Alderton et al., 2001). Following brain injuries, NO derived from either nNOS or iNOS plays a detrimental role (Iadecola et al., 1997; Mésenge et al., 1996; Parmentier-Batteur et al., 2001; Wada et al., 1999), whereas NO produced by eNOS has beneficial effects (Huang et al., 1996). Although iNOS is the primarily isoform responsible of NO involvement in the inflammatory processes, its genetically deletion in iNOS knock-out mice has shown to be either beneficial in brain ischemia (Iadecola et al., 1997; Zhao et al., 2000) or on the contrary detrimental in neurotrauma (Sinz et al., 1999). Therefore, long-term inhibition of iNOS is not necessarily beneficial and provides an evidence of the complexity in the design of neuroprotective strategies where exact timing and extent of inflammatory processes must be taken into account.

* Corresponding author. Tel.: +33 1 53 73 97 88; fax: +33 1 43 26 46 97.
E-mail address: mehrnaz.jafarian@univ-paris5.fr
(M. Jafarian-Tehrani).

0165-0270/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.jneumeth.2004.11.002

Several in vivo models of neuroinflammation have been described so far (Blond et al., 2002; Iravani et al., 2002; Kim et al., 2000; Matsuoka et al., 1999; Montero-Menei et al., 1996; Yamada et al., 1999), nevertheless none of them provides a model with parallel kinetic studies of acute inflammatory response, neurodegeneration and functional outcome. Therefore, the aim of the present study was to develop a rat model to study purely acute neuroinflammation on the histopathological, biochemical and functional outcomes for further mechanistic studies and evaluation of the potential neuroprotective agents. Therefore, we characterized a model using lipopolysaccharide (LPS), known as a strong iNOS inducer (Konsman et al., 1999; Stern et al., 2000; Wong et al., 1996), which was injected into the dentate gyrus of hippocampus. Histopathological and biochemical outcomes were evaluated as follows: spatiotemporal evolution of the inflammation and cell loss was studied from 6 h to 7 days following LPS injection. Neuroinflammation was evaluated by iNOS immunoreactivity, iNOS activity, NO production *via* NO end-product metabolites (nitrite plus nitrate, NO_x), and myeloperoxidase (MPO) activity. Neurodegeneration was assessed by cresyl violet staining, NeuN immunoreactivity, cNOS activity and astrogliosis by GFAP immunoreactivity. Finally, a sensorimotor neurological score was used to evaluate the functional outcome following LPS injection.

2. Materials and methods

2.1. Animals

Adult male Sprague-Dawley rats (Iffa-Credo, France) were maintained under standard laboratory conditions with controlled temperature and 12 h light/dark cycle and access to food and water ad libitum. Animal care and all experiments were in accordance with the French regulations and the European Communities Council Directive of November 24, 1986 (86/609/EEC) on the protection of animals for experimental use.

2.2. Animal model and experimental protocol

Rats weighing 300–350 g were anesthetized with chloral hydrate (400 mg/kg, i.p.) and placed on a stereotaxic frame. During surgery, animals were placed on a heating blanket system (Harvard Apparatus, UK) to maintain body temperature at $37.5 \pm 0.5\,^{\circ}C$. Intrahippocampal injection of lipopolysaccharide (LPS; *E. coli* serotype 0127:B8; Sigma, France) was performed as previously described (Louin et al., 2004). Briefly, the injection cannula (30G) was implanted unilaterally into the right dentate gyrus of the hippocampal formation at the stereotaxic coordinates (Paxinos and Watson, 1986), relatives to the bregma, as follows: 2.2 mm lateral, 3.8 mm posterior and 4 mm ventral from top of the skull. LPS (15 μg) was dissolved in 2 μl of sterilized physiological saline and was infused at a rate of 1 μl/min. The dose of LPS chosen in this study has previously been shown to induce iNOS protein and activity in vivo (Louin et al., 2004). Control rats received sterile physiological saline (2 μl) with the same protocol.

For immunohistochemistry (IHC) or NOS activity assays, rats were randomly assigned into naive ($n = 5$), saline- ($n = 2$–5 per each time point) or LPS-treated ($n = 5$ per each time point) groups at different times from 6, 15, 24, 48, 72 h to 7 days post-injection. Anesthetized rats were decapitated and brains were quickly removed and frozen in isopentane at $-40\,^{\circ}C$ for IHC studies. For NOS activity assays, hippocampus was dissected out on ice, quickly frozen and kept at $-40\,^{\circ}C$ until use.

For MPO activity or NO_x assay, rats were randomly assigned into three groups as follows: naive rats, saline- or LPS-treated groups at 24 h post-injection ($n = 6$–10 per group). Anesthetized rats perfused transcardially (for MPO activity), through the aorta with 200 ml saline at 100 mmHg of pressure in order to wash out the blood cells from the vasculature. Thereafter, rats were decapitated and the hippocampus was dissected out on ice, quickly frozen and kept at $-40\,^{\circ}C$ for MPO activity assay or processed online for NO_x assay.

2.3. Functional outcome assessment

A neurological examination was performed, after LPS or vehicle administration, by a single examiner blinded for the treatment following a grading scale previously described (Besson et al., 2003; Wahl et al., 1997). Briefly, contralateral sensorimotor functions were examined by assessing placing reactions (leg hanging and visual), grasping reflex (left forepaw and left hindpaw), and righting reflex (head tilted; left side and right side) in rats placed on a table. Rats were also examined for abnormal postures (thorax twisting and left forelimb flexion). The scores for each item were summed and used as a global neurological score. The maximum score was 9 for naive rats; the lower the neurological score the more severe the deficit.

2.4. Immunohistochemistry

Brain sections (20 μm) were cut every half-millimeter at six coronal planes from 2.8 to 5.3 mm posterior to the bregma at $-20\,^{\circ}C$ (cryostat Jung CM3000, Leica, Rueil-Malmaison, France). Sections were stained with cresyl violet to assess the hippocampal damage. Additional sections were processed for IHC to identify the cell subpopulation or to assess the neuroinflammation. Frozen sections were first dried, fixed in chilled acetone for 5 min and then incubated with 0.3% H_2O_2, 10% methanol in PBS (pH 7.4) for 10 min, followed by an incubation with a blocking solution containing 0.3% gelatin and 0.25% Triton X in PBS for 30 min. The primary antibodies used were monoclonal mouse anti-NeuN (1:100; Chemicon) to label neurons, monoclonal mouse anti-GFAP