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Adhesion molecules as potential targets for neuroprotection in a rodent model of Parkinson's disease

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ABSTRACT

Cell adhesion molecules might play an important role in the inflammatory mechanisms associated with neurodegeneration. We have previously observed, in rats, that subcutaneous injection of complete Freund's adjuvant (CFA), a pro-inflammatory agent that induces a peripheral inflammatory stimulus, reduces the nigrostriatal degeneration and microglial activation caused by stereotaxic injection of 6-hydroxydopamine (6-OHDA). Here we further investigated the effects of CFA in 6-OHDA-lesioned rats by evaluating the expression of selected adhesion molecules, both at central and peripheral levels.

Male, Sprague–Dawley rats received a subcutaneous injection of CFA followed, 10 days later, by intrastriatal injection of 6-OHDA. Animals were sacrificed at various time points and changes affecting intercellular (ICAM-1), vascular (VCAM-1), platelet endothelial (PECAM-1) and neural (NCAM-1) cell adhesion molecules were analyzed in striatum, ventral midbrain (containing the substantia nigra) and sera. Our results confirmed the protective effect of systemic CFA on 6-OHDA-induced nigrostriatal degeneration. Injection of 6-OHDA increased striatal ICAM-1 and PECAM-1 expression, while opposite changes (decreased expression) were detected in the ventral midbrain, particularly for VCAM-1 and NCAM-1. Pretreatment with CFA counteracted these changes. Nigrostriatal degeneration also affected peripheral immune function, with lesioned animals showing increased sPECAM levels with respect to intact animals. Also in this case, CFA pretreatment blocked the 6-OHDA induced increase of sPECAM.

Our findings confirm that a pre-existing, peripheral pro-inflammatory condition reduces the neuroinflammatory response and associated neurodegeneration provoked by centrally-administered 6-OHDA, with a mechanism that seems to involve selected adhesion molecules. The link between peripheral and central immune responses may, therefore, represent a target for new therapeutic strategies aimed at reducing the neuroinflammatory component associated with neurodegeneration.

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Introduction

Neuroinflammation has recently emerged as a crucial complement of neurodegeneration in Parkinson's disease (PD) (Brochard et al., 2009; McGeer and McGeer, 2008; McGeer et al., 1988). Inflammatory changes, commonly detected in PD brains, have been reproduced in various animal models of PD. In these models, the nigrostriatal degeneration caused by administrations of neurotoxins such as 6-hydroxydopamine (6-OHDA), rotenone or 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is invariably associated with glial activation (Armentero et al., 2006b; Carta et al., 2009; Frau et al., 2011; Sherer et al., 2003) and, in some cases, lymphocyte infiltration (Brochard et al., 2009).

Adhesion molecules (AMs) are members of the immunoglobulin gene super-family that mediate communication among adjacent endothelial cells, as well as between endothelium and leukocytes. At the peripheral level, AMs allow homing of effector cells to sites of inflammation; in the brain, AMs contribute to the regulation of brain plasticity, neuronal survival and integrity of the blood–brain barrier (BBB). Intercellular (ICAM-1), vascular (VCAM-1), platelet endothelial (PECAM-1) and neural (NCAM-1) cell adhesion molecules are expressed on activated glial cells (Akiyama et al., 1993; Miklossy et al., 2006; Xu et al., 2010) and inflamed BBB (Bell and Perry, 1995)

Abbreviations: 6-OHDA, 6-hydroxydopamine; AM, adhesion molecule; BBB, Blood–brain barrier; CFA, complete Freund's adjuvant; GDNF, glial derived neurotrophic factor; ICAM-1, intercellular cell adhesion molecules; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; NCAM-1, neural cell adhesion molecules; PD, Parkinson's disease; PECAM-1, platelet endothelial cell adhesion molecules; SNc, substantia nigra pars compacta; VCAM-1, vascular cell adhesion molecules; VMB, ventral midbrain.

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during neuroinflammatory reactions. In particular, a large number of ICAM-1-positive reactive astrocytes have been reported in the substantia nigra pars compacta (SNc) of PD patients (Brochard et al., 2009; Miklossy et al., 2006), and have also been detected in MPTP-treated mice (Brochard et al., 2009) and monkeys (Miklossy et al., 2006). To date, however, little is known about the contribution of AMs to disease progression.

Earlier studies have indicated that peripheral inflammation may modulate the development of toxin-induced neuronal damage (Armentero et al., 2006b; Kurkowska-Jastrzebska et al., 2005, 2009). We have shown, for example, that pre-treatment with complete Freund's adjuvant (CFA) – commonly used to enhance host immune response to vaccinal antigen stimulation – significantly reduces the nigrostriatal damage caused by 6-OHDA in rats (Armentero et al., 2006b). Such protective effect was paralleled by reduced microglial activation and enhanced expression of specific trophic factors and cytokines at the site of neurodegeneration.

To confirm and extend these findings, in this study we further analyzed CFA effects by assessing how treatments with 6-OHDA and/or CFA affect the expression of PECAM-1, ICAM-1, VCAM-1 and NCAM-1, in the brain areas specifically undergoing 6-OHDA induced degeneration, as well as at peripheral level (serum).

Material and methods

Male Sprague Dawley rats (200–225 g; Charles River, Italy) were used. All procedures were in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and were approved by the local Animal Care Committee. CFA containing *tuberculosis* (Sigma) was resuspended in an oil:PBS emulsion (1:1) and injected subcutaneously (s.c.) into each flank of the animals (0.1 mL/flank). Control animals received a s.c. injection of PBS. All animals receiving the CAF/PBS emulsion showed typical signs of local inflammation, within a few days from injections, which was still present at the time of sacrifice. Ten days after the s.c. injection, animals were anesthetized (50 mg/kg sodium-thiopental) and received a stereotaxic injection of 6-OHDA (20 μg/3 μL saline containing 0.2 mg/mL ascorbic acid) or vehicle, into the right striatum (1 mm anterior, 3.0 mm lateral and 5.0 mm ventral) – with respect to bregma and dura (Paxinos and Watson, 1998) as described before (Armentero et al., 2006a).

At various time points after CFA injection (see Fig. 1) animals were sacrificed by decapitation and serum, obtained from trunk's blood, was stored at −80 °C. Animals from each treatment group were arbitrarily subdivided in two subgroups and brains were either frozen intact, for evaluation of 6-OHDA-induced lesion, or divided in two using a cold Coronal Brain Matrix (2 Biological Instruments, Italy), for AM analysis.

Loss of tyrosine hydroxylase (TH)-positive terminals and neurons was evaluated following immunohistochemical staining on coronal sections containing striatum or SNc, as described before (Armentero et al., 2006a).

For AM analysis, striatum and ventral midbrain (VMB) area, containing the SNc, were dissected out and homogenized, as previously described (Armentero et al., 2006a); tissue homogenates were analyzed by western blot using specific primary antibodies against β–actin, VCAM-1, ICAM-1 and PECAM-1 (all from Santa Cruz) and NCAM-1 (Sigma). IRDye800 and IRD700 anti-rabbit or anti-mouse secondary antibodies were used for detection (Li-Cor Biosciences). Quantitative analysis was performed using a near Infrared scanner (Odyssey Imager, Li-Cor) equipped with a dedicated software. All values were normalized to actin expression. Specificity of the primary antibodies were controlled using the respective immunizing peptides (blocking peptide). Briefly, each antibody was incubated ON at 4 °C, in 500 μL PBS, in the presence or absence of a 10 fold excess of blocking peptide. The antibody/peptide mixture was then diluted in blocking buffer to obtain the correct antibody concentration and used for subsequent analysis.

Serum levels of soluble AMs were measured using colorimetric ELISA Kits, according to the manufacturer's instructions (Diaclone, France). Detection limits were 0.17 ng/ml (sPECAM-1), 0.6 ng/ml (sVCAM-1) and 0.1 ng/ml (sICAM-1).

All values are expressed as mean ± sem. Comparisons between groups were carried out using the analysis of variance (ANOVA) coupled with the Tukey's post-hoc, using a dedicated software (Prism 3 software, GraphPad Software, USA). The minimum level of statistical significance was set at $p < 0.05$.

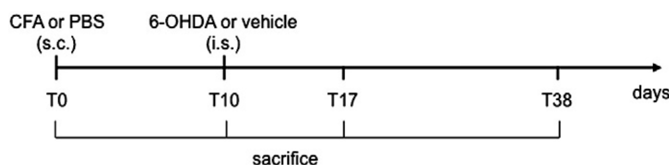

Fig. 1. Schematic representation of the experimental paradigm. Animals were injected sub-cutaneously (s.c.) with CFA on both flanks or PBS and received, 10 days later, an intrastriatal (i.s.) injection of 6-OHDA or vehicle. Animals were sacrificed immediately (T0) or 10 (T10), 17 (T17) and 38 days (T38) after CFA treatment.

Results

Intrastriatal 6-OHDA injection induced progressive loss of dopaminergic terminals, in the striatum (Fig. 2A), and cell bodies in the SNc (Fig. 2B). Pre-treatment with CFA significantly reduced toxin-induced degeneration in both nuclei (Figs. 2A and B).

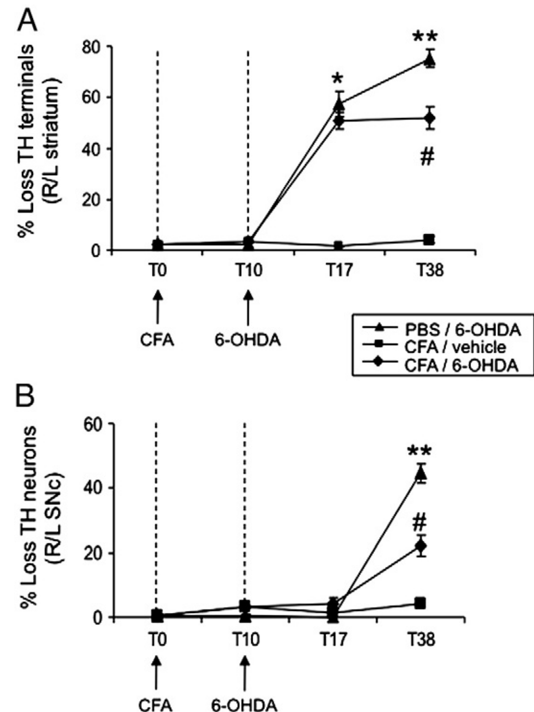

Fig. 2. CFA pre-treatment reduces 6-OHDA induced nigrostriatal degeneration. (A) Loss of TH-positive dopaminergic terminals in the ipsilateral striatum. Values (mean ± sem) are expressed as the percentage (%) of the striatal volume deprived of TH immunoreactivity with respect to the entire striatal volume in the injected hemisphere. (B) Loss of TH-positive dopaminergic neurons in the ipsilateral SNc. Values (mean ± sem) are expressed as the percentage (%) of TH-positive neurons loss in the right, lesioned hemisphere, compared to the left, intact hemisphere. * $p < 0.0001$ PBS/6-OHDA T17 and CFA/6-OHDA T17 vs respective T10; ** $p < 0.001$ PBS/6-OHDA T38 vs T17, # $p < 0.005$ CFA/6-OHDA T38 vs PBS/6-OHDA T38. Vertical dotted lines indicate respective time of CFA (T0) and 6-OHDA injection (T10). Horizontal dotted line represents the mean control value.

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