Available online at www.sciencedirect.com
SCIENCE DIRECT®
Neuroscience Letters 383 (2005) 44–48
Neuroscience Letters
www.elsevier.com/locate/neulet

Granulocyte colony-stimulating factor is not protective against selective dopaminergic cell death in vitro

Carmen Henze, Thomas Lescot, Sabine Traver, Bénédicte Salthun-Lassalle, Etienne C. Hirsch, Patrick P. Michel, Andreas Hartmann *

INSERM U 679 (former U 289), Experimental Neurology and Therapeutics, Centre d'Investigation Clinique, Hôpital de la Salpêtrière, 47 Boulevard de l'Hôpital, 75651 Paris Cedex 13, France

Received 17 January 2005; received in revised form 21 March 2005; accepted 21 March 2005

Abstract

In the present study, we evaluated the potential neuroprotective effect of granulocyte colony-stimulating factor (G-CSF), a hematopoietic growth factor in two different culture models in which dopaminergic (DA) neurons die selectively: first, in a culture model in which death of DA neurons occurs spontaneously and second, in a toxin-based paradigm, the in vitro 1-methyl-4-phenylpyridinium model of PD. In neither of the two models, a treatment with G-CSF, could prevent or halt the progressive neurodegeneration. However, we cannot rule out that G-CSF might exert neuroprotective or even deleterious effects in in vivo models of PD, based on the significant increase in the number of microglial cells observed after G-CSF treatment.

Keywords: Parkinson's disease; Granulocyte colony-stimulating factor; 1-Methyl-4-phenylpyridinium

Parkinson's disease (PD) is a common neurodegenerative disorder and is associated with a progressive loss of dopaminergic (DA) neurons in the substantia nigra pars compacta (SNpc). The ensuing diminution of the dopamine (DA) concentration in the striatum induces the characteristic disease symptoms of bradykinesia/akinesia, rest tremor and rigidity.

Granulocyte colony-stimulating factor (G-CSF), a key hematopoietic growth factor of the myeloid lineage, has been used for more than 10 years in the treatment of neutropenia as well as for bone marrow reconstitution and stem cell mobilization. Recently, evidence has emerged that G-CSF exerts neuroprotective effects. Zavala et al. [15] demonstrated that G-CSF exerts protective effects on the course of experimental allergic encephalomyelitis, a murine model for multiple sclerosis: animals treated with G-CSF displayed only limited demyelination and almost no inflammation. Also, a recent paper reported that G-CSF has neuroprotective effects in an animal model of focal cerebral ischemia [14], and confirmed the presence of G-CSF receptors (G-CSFR) in the rat central nervous system (CNS), both on glial cells and neurons. G-CSF itself seems to be predominantly secreted by astroglia within the CNS [6]. The potential neuroprotective effect of G-CSF in stroke, and multiple sclerosis could—inter alia—be mediated by its anti-inflammatory properties, which is relevant in PD [7]. In vivo, G-CSF may also enhance neurogenesis by a yet unknown mechanism [1].

Based on this rationale, we evaluated the potential therapeutic effect of G-CSF in two different culture models: first, in rat embryonic primary mesencephalic cell cultures in which DA neurons die spontaneously by apoptosis [9] and second, in rat embryonic primary mesencephalic cell cultures exposed to the DA toxin 1-methyl-4-phenylpyridinium (MPP^+) [4]. Furthermore, we analysed the expression pattern of G-CSFR by fluorescent double-labelling experiments as well as the effect of G-CSF on cell proliferation.

Animal treatment was performed according to the guidelines of the local animal care and use committee. Cultures of postmitotic DA neurons [13] were prepared from the ventral mesencephalon of embryonic day 15.5 Wistar rat embryos (Breeding Center Janvier, Le Genest St. Isle, France) as described [9]. All culture experiments were done at least in trip-

* Corresponding author. Tel.: +33 1 42 16 22 02; fax: +33 1 44 24 36 58.
E-mail address: hartmann@ccr.jussieu.fr (A. Hartmann).

0304-3940/$ – see front matter © 2005 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.neulet.2005.03.041

licate. Per experimental condition, an average of three wells were used, that means at least nine wells in total. In the culture model of spontaneous cell death, but not the MPP^+ culture model, the N5 medium was additionally supplemented with 5 mM glucose. Cultures were fed daily by replacing 300 of the 500 μl of medium in the culture wells. Cytosine arabinoside (ara-C; Sigma-Aldrich)-treated cultures were maintained in the presence of 1 μM the NMDA receptor antagonist MK-801 (Sigma-Aldrich).

Cultures were treated with G-CSF for 10 days with 0.1, 1 or 10 ng/ml G-CSF (PeproTech EC LTD, London, UK) based on the study by Schäbitz et al. [14]. As a positive control to promote cell survival, ara-C was added in some wells at a concentration of 2 μM during the first 2 days in vitro (DIV). Ara-C exerts a neuroprotective effect by eliminating of dividing glial cells from the cultures [11]. To study the effect of G-CSF on cell proliferation, the number of cells incorporating [*methyl*-^{3}H]thymidine [2] into their DNA was quantified on DIV6 under the same treatment conditions. As a positive control, epidermal growth factor (EGF; PeproTech EC), which stimulates the division of astrocytes [8], was administered daily at a concentration of 20 ng/ml.

After 5 days in culture, cells were treated with 0.1, 1 or 10 ng/ml G-CSF. Thirty minutes later, MPP^+ (Sigma-Aldrich) was added for 48 h at a concentration of 1 μM as described [10].

Morphological preservation of DA neurons was determined by tyrosine hydroxylase (TH) immunocytochemistry. The cultures were incubated with a monoclonal anti-TH antibody (1:5000; DiaSorin, Stillwater, MN, USA). Cultures were then incubated with a biotinylated anti-mouse IgG_{2a} (1:100; Vector Laboratories, UK) followed by a preformed streptavidin–horseradish peroxidase complex (1:100; Vector Laboratories). The peroxidase substrate was diaminobenzidine (0.5 mg/ml). The expression of G-CSFR was analysed in fluorescent double-labelling experiments by incubating cultures with a polyclonal rabbit anti-G-CSFR antibody (1:400; Santa Cruz Biotechnology). G-CSFR was revealed using an anti-rabbit antibody coupled to CY3 (1:400; Sigma-Aldrich). The cultures were then incubated with monoclonal antibodies for microtubule-associated protein 2 (1:250; MAP-2; Sigma-Aldrich) to identify neuronal cells regardless of their neurotransmitter phenotype, for TH (1:5000; DiaSorin) to stain DA neurons, or for glial fibrillary acidic protein (GFAP, DAKO

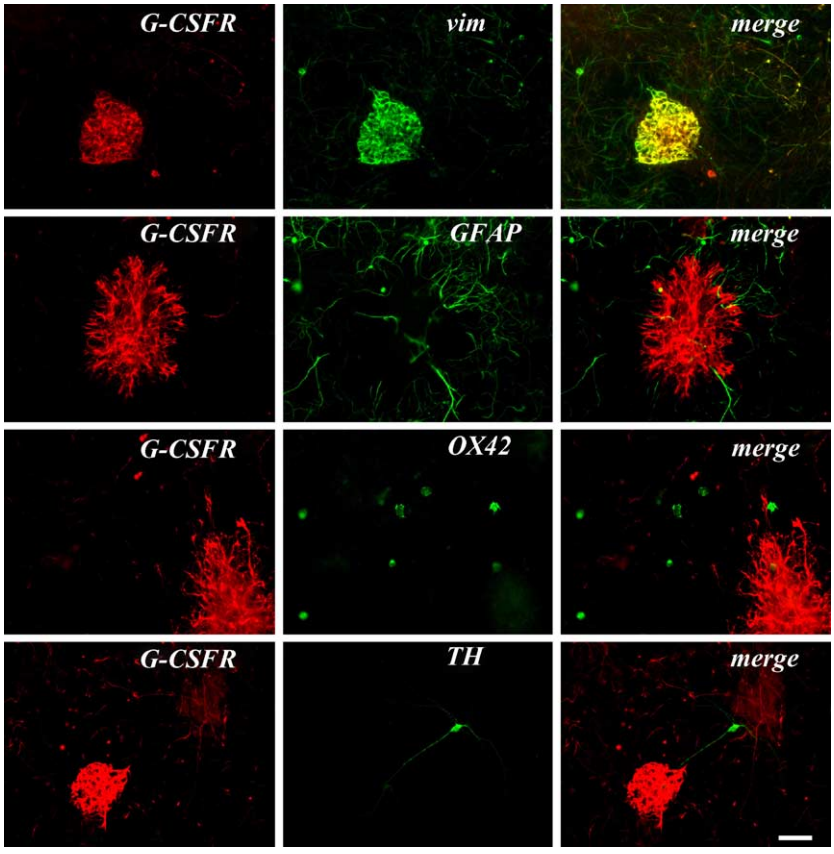

Fig. 1. G-CSFR immunoreactive cells are present in mesencephalic cultures. In fluorescent double-labelling experiments, G-CSFR immunoreactivity was detectable on vimentin-positive (vim) cells, whereas G-CSFR labeling was absent from GFAP-, OX42- or TH-positive cells. Note the flame-like morphology of the cell clusters which are double-positive for G-CSFR and vimentin. Detection of G-CSFR-positive cells was done using a CY3 conjugate (red) and vimentin-, GFAP-, OX42- or TH-positive cells using an Alexa Fluor® 488 antibody (green), respectively. Scale bar: 50 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.)