Available online at www.sciencedirect.com

SCIENCE DIRECT

Experimental Neurology 192 (2005) 167–177

Experimental Neurology

www.elsevier.com/locate/yexnr

Macrophage colony stimulating factor (M-CSF) protects spiral ganglion neurons following auditory nerve injury: morphological and functional evidence

Akinori Yagihashi, Tetsuji Sekiya*, Shigehru Suzuki

Department of Neurosurgery, Hirosaki University School of Medicine, 5 Zaifu-cho, Hirosaki, 036-8216, Japan

Received 25 November 2003; revised 2 September 2004; accepted 19 October 2004
Available online 21 January 2005

Abstract

Because hearing disturbance due to auditory nerve dysfunction imposes a formidable burden on human beings, intense efforts have been expended in experimental and clinical studies to discover ways to restore normal hearing. However, the great majority of these investigations have focused on the peripheral process side of bipolar auditory neurons, and very few trials have focused on ways to halt degenerative processes in auditory neurons from the central process side (in the cerebellopontine angle). In the present study, we investigated whether administration of macrophage colony-stimulating factor (M-CSF) could protect auditory neurons in a rat model of nerve injury. The electrophysiological and morphological results of our study indicated that M-CSF could ameliorate both anterograde (Wallerian) and retrograde degeneration in both the CNS and PNS portions of the auditory nerve. We attribute the success of M-CSF therapy to the reported functional dichotomy (having the potential to cause both neuroprotective and neurotoxic effects) of microglia and macrophages. Whether the activities of microglia/macrophages are neuroprotective or neurotoxic may depend upon the nature of the stimulus that activates the cells. In the present study, the neuroprotective effects of M-CSF that were observed could have been due to M-CSF we administered and to M-CSF released from endothelial cells, resident cells of the CNS parenchyma, or infiltrating macrophages. Another possibility is that M-CSF ameliorated apoptotic auditory neuronal death, although this hypothesis remains to be proved in future studies.

Keywords: Apoptosis; Auditory nerve; Hearing; Macrophage colony stimulating factor; Nerve degeneration; Spiral ganglion cell; TUNEL

Introduction

Auditory neurons are bipolar neurons with peripheral (hair cell side) and central (cochlear nucleus side) processes (Fig. 1). Both of these processes are vulnerable to various insults, and damage to either process can lead to auditory nerve dysfunction and hearing disturbance (Geisler, 1998; Sekiya et al., 2000a,b,c, 2001; Shimamura et al., 2002; Spoendlin, 1987). As hearing disturbance due to auditory nerve dysfunction imposes a formidable burden on humans, enormous efforts have been expended to clarify the pathophysiological mechanisms responsible for hearing disturbance due to auditory nerve injury and to restore lost hearing (Altschuler et al., 1999; Fredelius, 1988; Kopke et al., 1996; Miller et al., 1997). However, such efforts have been focused mainly on the peripheral processes of auditory neurons; hearing disturbance due to central process injury has rarely been investigated (Fredelius, 1988; Geschwind et al., 1996; Sekiya et al., 2000a; Staecker et al., 1998). Because the central process of the auditory nerve is, as described below, equally indispensable to hearing, this

Abbreviations: ABI, auditory brainstem implant; BAEP, brainstem auditory evoked potential; CAP, compound action potential of the auditory nerve; CNS, central nervous system; CP, cerebellopontine; CR, compression-recording; IAM, internal auditory meatus; M-CSF, macrophage colony stimulating factor; PBS, phosphate-buffered saline; PNS, peripheral nervous system; SGC, spiral ganglion cell; TUNEL, terminal deoxynucleotide transferase-mediated-dUTP (2′-deoxyuridine 5′-triphosphate) nick end labeling.

* Corresponding author. Department of Otolaryngology, Head and Neck Surgery, Kyoto University, Graduate School of Medicine, Sakyou-ku, Kyoto, 606-8507, Japan. Fax: +75 751 7225.
E-mail address: tsekiya@ent.kuhp.kyoto-u.ac.jp (T. Sekiya).

0014-4886/$ - see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.expneurol.2004.10.020

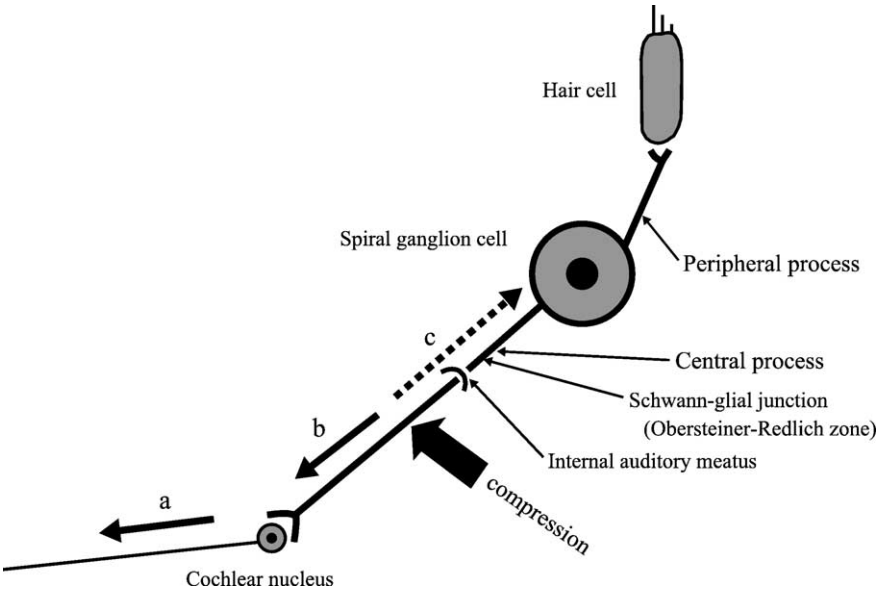

Fig. 1. Schematic representation of procedure for compression of the central process of the auditory nerve in the cerebellopontine angle (large black arrow). a, b, and c indicate transneuronal, anterograde (Wallerian), and retrograde degeneration of the auditory nerve.

unexplored area of auditory nerve research should be investigated. First, the central processes are at risk during surgery to remove a vestibular schwannoma (acoustic neurinoma) because they are exposed in the cerebellopontine (CP) angle. Direct traumatic injury to these processes may be difficult to avoid, with the result that CP angle surgery poses a significant risk to patients' hearing (Hatayama et al., 1999; Sekiya et al., 2000a,b). Thus, patients with bilateral vestibular schwannomas, such as those with neurofibromatosis II (NF2), are at particular risk of total hearing loss following surgery (Eldridge and Parry, 1992; Sekiya et al., 2000a). Second, cochlear implant surgery cannot be successful unless there are sufficient numbers of viable spiral ganglion cells (SGCs) and their central processes (Miura et al., 2002). Third, even though auditory brainstem implant (ABI) surgery can be performed (to stimulate the cochlear nucleus directly in the brainstem) to restore some hearing to patients with bilateral central hearing loss (such as those who have undergone bilateral vestibular schwannoma removal or who have bilateral auditory nerve aplasia) (Colletti et al., 2002; Marangos et al., 2000; Matthies et al., 2000; Otto et al., 2001), its success undoubtedly depends on the number of surviving cochlear nucleus cells and higher auditory relay nuclei (Sekiya et al., 2000a). Thus, it is desirable to preserve as many central processes of auditory neurons as possible, because injury to central processes not only causes auditory nerve degeneration but also transneuronal degeneration of the cochlear nucleus cells and cells in the higher auditory nuclei (Morest et al., 1997; Willott and Lu, 1982) (Fig. 1a).

Macrophage colony-stimulating factor (M-CSF) is one of the colony-stimulating factors, along with granulocyte-CSF, granulocyte/macrophage-CSF, and multi-CSF (IL-3)(Barreda et al., 2004). These substances are cytokines that have been shown to have central roles in the process of hematopoiesis and modulation of blood cell functional responses, and in the maintenance of homeostasis and overall immune competence (Flanagan and Lader, 1998). M-CSF is synthesized by a variety of cell types, including endothelial cells, fibroblasts, bone marrow stromal cells, and osteoblasts (Barreda et al., 2004; Flanagan and Lader, 1998; Lenarz et al., 2002). The biological effects of M-CSF are mediated via receptors with high affinity for M-CSF (Barreda et al., 2004; Flanagan and Lader, 1998; Guilbert and Stanley, 1980). The M-CSF receptors (M-CSFRs) belong to the class III tyrosine kinase family of receptors and are expressed primarily on cells in the macrophage lineage (Guilbert and Stanley, 1980). However, following injury to, or in pathological conditions, M-CSFRs can be found in these nervous in astrocytes or neurons (Wang et al., 1999). Clinically, administration of M-CSF has been found to shorten periods of neutropenia and thrombopenia following chemotherapy in patients with acute myelogenous leukemia (Motoyoshi, 1998) and has proved effective in the control of invasive fungal infections in patients undergoing bone marrow transplantation (Nemunaitis et al., 1998).

Despite the protective effects of M-CSF in these clinical situations and the fact that M-CSF receptors are expressed on astrocytes and neurons following injury, to the best of our knowledge, no study has yet been performed to investigate the effectiveness of M-CSF to prevent pathological processes related to trauma to the nervous system. We therefore conducted the study reported here to investigate the effectiveness of M-CSF in protecting auditory neurons injured directly in the CP angle. Our results provide the first clear evidence that a pharmacologic agent in clinical use with an established safety profile (Jakubowski et al., 1996; Masaoka et al., 1990; Mizutani et al., 2003) is