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BRAIN RESEARCH

Research Report

Monocytes deliver bioactive nerve growth factor through a brain capillary endothelial cell-monolayer *in vitro* and counteract degeneration of cholinergic neurons

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ARTICLE INFO

Article history:
Accepted 21 November 2009
Available online 11 December 2009

Keywords:
Neuroprotection
Nerve growth factor
Alzheimer
Transmigration
NGF secreting monocyte

ABSTRACT

Alzheimer's disease is an age-dependent brain disorder, characterized by progressive memory deficits and cognitive decline and loss of cholinergic neurons. Nerve growth factor (NGF) is the most potent protein to protect cholinergic neurons against degeneration. However, problems of delivery to the brain limit the therapeutical use of NGF. The aim of the present study was to test, if primary rat monocytes can be loaded with recombinant NGF and pass an *in vitro* monolayer of brain capillary endothelial cells (BCEC), release NGF, and support the cholinergic neurons in an organotypic brain slice model. Monocytes were isolated from rat blood by negative magnetic selection, loaded with recombinant NGF using Bioporter™. The monocytes adhered and migrated through an *in vitro* rat BCEC-monolayer. NGF released at the basolateral side counteracted degeneration of cholinergic basal nucleus of Meynert neurons. In conclusion, our present study shows a proof-of-principle, that primary monocytes secreting NGF might be useful tools to deliver NGF into the brain, however, further *in vivo* studies are necessary.

1. Introduction

Alzheimer's disease (AD) is an age-dependent neurodegenerative disease characterized by progressive loss of cognitive functions and by characteristic pathological changes in the brain, such as extracellular aggregates of beta-amyloid and hyperphosphorylation of tau proteins. The cholinergic neurons of the nucleus basalis of Meynert (nBM) provide the major cholinergic innervation to the neocortex and are essential for cortical cognitive functions and memory. Cholinergic neurons degenerate in AD and lack of cortical acetylcholine correlates with cognitive decline (Francis et al., 1999; Schliebs and Arendt, 2006).

Nerve growth factor (NGF) is the most potent neuroprotective protein for cholinergic neurons in the brain (Levi-Montalcini, 1987). It is well established that in adult rats and primates recombinant exogenous NGF prevents the degeneration of choline acetyltransferase positive (ChAT+) neurons *in vitro* and *in vivo* (Counts and Mufson, 2005; Tuszynski and Blesch, 2004; Lapchak, 1993; Perry, 1990; Dreyfus, 1989; Hefti and Will, 1987). Clinical studies where NGF was infused into the ventricle of an Alzheimer patient revealed transient but

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doi:10.1016/j.brainres.2009.11.062

slight improvements of symptoms but some severe side effects (Olson et al., 1992). Recently genetically engineered skin cells secreting NGF were transplanted into the basal nucleus of Meynert (nBM) of AD patients by Tuszynski and colleagues (2005). However, the clinical use of NGF in humans is limited because (1) NGF must be delivered continuously over years due to the progressive nature of the degeneration of the disease and (2) NGF should be delivered as early as possible at the onset of the disease to protect intact remaining cholinergic neurons from degeneration. Furthermore, as it is expected that 80 million people will suffer from AD worldwide within the next 50 years, invasive surgical methods will not be possible.

Thus, research strategies try to explore novel non-invasive delivery methods of NGF to the brain: intranasal or intraocular NGF application (Capsoni et al., 2009), non-proteinergic NGF agonists which pass the blood–brain barrier (BBB), NGF coupled to BBB-passing molecules, such as e.g. transferrin receptors (Granholm et al., 1994) or application of NGF-loaded microspheres (Gu et al., 2009). Alternatively to direct NGF delivery, a cell based approach may be suitable to use cells as a vehicle for NGF transport across the BBB. Recently, Danielyan et al. (2009) reported that intranasal delivery of cells might provide a new non-invasive method for cell delivery to the CNS. In our research group, we are interested to test if monocytes, loaded with NGF are useful to deliver NGF into the brain. We have previously shown that NGF-loaded monocytes transplanted into the brain can protect cholinergic neurons against degeneration (Zassler and Humpel, 2006). Monocytes are pluripotent immune cells circulating in the blood and are the precursor cells of the macrophages and microglia in the CNS. They are formed in the bone marrow, are released into the blood and migrate into most organs where they differentiate into specialized organ macrophages (Van Furth and Cohn, 1968; Volkman, 1970). In the CNS they can differentiate into resting microglia, which become activated by certain stimuli e.g. after neurodegeneration (Gehrmann et al., 1995; Kreutzberg, 1996; Perry et al., 1995; Stoll and Jander, 1999; Wilms et al., 1997). It is well established that monocytes can transmigrate through an *in vitro* BBB and migrate to lesion sites, where they can differentiate into microglia-like cells or macrophages (Moser and Humpel, 2007; Giri et al., 2002; Ifergan et al., 2008; Leone et al., 2006). Especially, neurodegeneration or inflammation recruits monocytes to lesion sites in the brain (D'Mello et al., 2009; Floris et al., 2002). Thus, a transmigration strategy may provide a potential non-invasive cell based protein delivery into the brain and may offer a possible cure approach for certain CNS diseases.

The aim of the present study was to explore if primary rat monocytes can be loaded with recombinant NGF, transmigrate through an *in vitro* brain capillary endothelial cell (BCEC)-monolayer (a simple *in vitro* BBB), deliver NGF and counteract degeneration of cholinergic nBM slices in organotypic brain slices.

2. Results

To explore the effects of NGF secreting monocytes we have developed a simple *in vitro* system, which consists of a small 3 μm pore membrane insert containing a full confluent monolayer of BCEC and a large 0.4 μm pore membrane insert on which organotypic brain slices are cultured with the conditioned medium after transmigration (Fig. 1).

2.1. *BCEC-monolayer and diffusion assay*

In order to characterize a full BCEC-monolayer, the cells were stained with F-actin phalloidin (Fig. 2A) and nuclear DAPI (Fig. 2B). When fluorescein (10 ng/100 μl) or NGF (3 ng/100 μl) was applied to the apical side of a 3 μm insert with or without a BCEC-monolayer, a time dependent diffusion was seen (Fig. 2). The diffusion of fluorescein was not different with or without a BCEC-monolayer (Fig. 2C), while the diffusion of NGF was reduced by the BCEC-monolayer (Fig. 2D).

2.2. *Primary monocytes and labelling*

In order to characterize rat monocytes, isolated cultured primary rat monocytes were immunohistochemically stained against CD68 (Fig. 3A). The CD68 staining was cytoplasmatic as visualized by DAPI co-staining (Figs. 3B and C). Among the isolated immunohistochemical stained monocytes 97±1% (n=10) were CD68 positive. For some experiments isolated primary rat monocytes were labelled with the dye PKH67 Green Fluorescent Cell Linker which

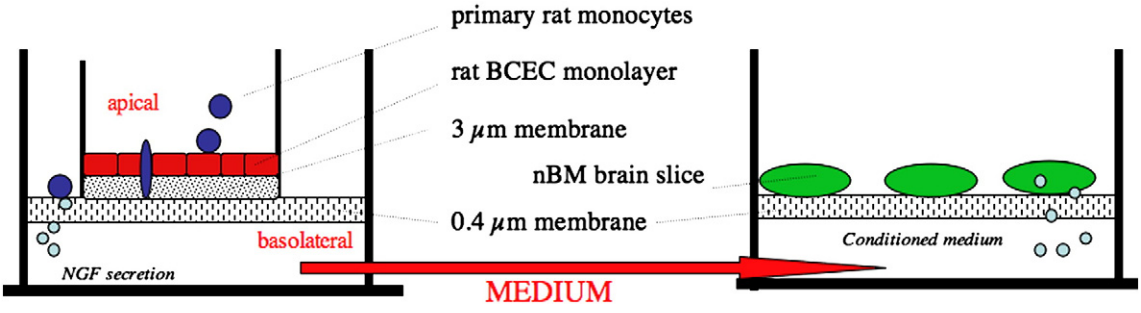

Fig. 1 – Scheme of the experimental set up: a large 30 mm 0.4 μm pore insert was placed into a 6-well plate with 1.3 ml medium (basolateral). Then a small 12 mm 3 μm pore insert (without feet) with a confluent BCEC-monolayer was placed onto the large insert. Monocytes or medium with or without NGF were applied into the small insert onto the BCEC-monolayer (apical side). After 18 h the small insert was removed, fixed and analyzed and the medium collected and used for culturing of brain slices on a large 0.4 μm membrane insert.