available at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/brainres

BRAIN RESEARCH

Research Report

Motoneuronotrophic factor analog GM6 reduces infarct volume and behavioral deficits following transient ischemia in the mouse

Jin Yu[a], *Hong Zhu*[a], *Dorothy Ko*[b], *Mark S. Kindy*[a,c,d,*]

[a]*Department of Neurosciences, Medical University of South Carolina, 173 Ashley Avenue, BSB 403, Charleston, SC 29425, USA*
[b]*Genervon Biopharmaceuticals, Montebello, CA, USA*
[c]*Ralph H. Johnson VA Medical Center, Charleston, SC 29401, USA*
[d]*Neurological Testing Service, Inc., Charleston, SC 29425, USA*

ARTICLE INFO

Article history:
Accepted 12 August 2008
Available online 29 August 2008

Keywords:
Stroke
Trophic factor
Middle cerebral artery occlusion
Mouse
Neuroprotection

ABSTRACT

Motoneuronotrophic factor (MNTF) is an endogenous neurotrophin that is highly specific for the human nervous system, and some of the observed effects of MNTF include motoneuron differentiation, maintenance, survival, and reinnervation of target muscles and organs. MNTF is a neuro-signaling molecule that binds to specific receptors. Using *In Silico* Analysis, one of the active sites of MNTF was identified as an analog of six amino acids (GM6). The effect of chemically synthesized GM6 on ischemic stroke was studied in the middle cerebral artery occlusion (MCAo) mouse model. Mice were subjected to 1 hur of ischemia followed by 24 h of reperfusion. Mice were injected intravenously with a bolus of GM6, at various doses (1 and 5 mg/kg) immediately after the start of reperfusion and examined for changes in physiological parameters, neurological deficits and infarct volume. GM6 was able to penetrate the blood brain barrier, and at both 1 and 5 mg/kg showed a significant protection from infarct damage, which translated to improvement of neurological deficits. Administration of GM6 demonstrated no changes in HR, BP, pO_2, pCO_2, or pH. A significant increase over the control group in CBF after reperfusion was observed with GM6 administration, which helped to mitigate the ischemic effect caused by the blockage of blood flow. The time window of treatment was assessed at various times following cerebral ischemia with GM6 demonstrating a significant protective effect up to 6–12 h post ischemia. In addition, GM6 increased neurogenesis, and decreased apoptosis and inflammation in the mouse brain following cerebral ischemic injury. These data suggest that GM6 is neuroprotective to the brain following IV injection in the mouse model of MCAo.

Published by Elsevier B.V.

* *Corresponding author*. Department of Neurosciences, Medical University of South Carolina, 173 Ashley Avenue, BSB 403, Charleston, SC 29425, USA. Fax: +1 843 876 5099.
E-mail address: kindyms@musc.edu (M.S. Kindy).

Abbreviations: MNTF, motoneuronotrophic factor; MCAo, middle cerebral artery occlusion; CBF, cerebral blood flow; HR, heart rate; BP, blood pressure

0006-8993/$ – see front matter. Published by Elsevier B.V.
doi:10.1016/j.brainres.2008.08.053

1. Introduction

Neuronotrophic factors (NTFs) are a specialized group of proteins which function to promote the survival, growth, maintenance, and functional capabilities of selected populations of neurons (Barde, 1988, 1989; and Thoenen and Edgar, 1985). Studies have demonstrated that neuronal death occurs in the nervous systems of vertebrates during certain periods of growth and development (Burek and Oppenheim, 1996; Mennerick and Zorumski, 2000; Sendtner et al., 2000; Buss and Oppenheim, 2004). However, the addition of soluble neuronal trophic factors from associated target tissues serves to mitigate this phenomenon of neuronal death (Chau et al., 1990; Kuno, 1990; Oppenheim, 1989; Krieglstein et al., 2002; Hennigan et al., 2007).

In the vertebrate neuromuscular system, the survival of embryonic motoneurons has been found to be dependent upon specific trophic substances derived from the associated developing skeletal muscles. Skeletal muscles have been shown, by both *in vivo* and *in vitro* studies, to produce substances which are capable of enhancing the survival and development of motoneurons by preventing the embryonic motoneurons from degeneration and subsequent, natural cellular death (O'Brien and Fischbach, 1986; Hollyday and Hamburger, 1976). Similarly, several investigators have reported that chick and rat skeletal muscles possess certain trophic factors which can prevent the natural cellular death of embryonic motoneurons both *in vivo* and *in vitro* (McManaman et al., 1988; Oppenheim and Haverkamp, 1988; Smith et al., 1986). These skeletal muscle derived neuronotrophic factors have been demonstrated to be functionally different from other trophic factors such as Nerve Growth Factor (NGF), Ciliary Ganglion Neurotrophic Factor (CNTF), Brain-Derived Neurotrophic Factor (BDNF), and Retinal Ganglion Neurotrophic Factor (RGNTF) (Edgar, 1985; Levi-Montalcini, 1982; Varon et al., 1988; Barde, 1989; McManaman et al., 1990; Chau et al., 1991a,b).

More recently, the isolation and characterization of two motoneuronotrophic factors from rat muscle tissue with apparent molecular weights of 35 kDa and 22 kDa have been reported (Chau et al., 1992). The 35 kDa protein was defined as motoneuronotrophic factor 1 (MNTF1) and the apparent 22 kDa protein as motoneuronotrophic factor 2 (MNTF2). These two trophic factors have been demonstrated *in vitro* to support the growth and/or regeneration of both isolated anterior horn motoneurons and spinal explants of rat lumber spinal cord. A number of studies have demonstrated the efficacy of these MNTFs in various rat nerve systems, including the peripheral sciatic nerve, the peripheral musculocutaneous nerve, the cranial facial nerve, the cranial hypoglossal nerve, and the portion of the spinal cord that controls muscles in the neck, chest and upper limbs (Zhou et al., 1992, 1993; Chau et al., 1992; Wang et al., 1995). In the hemisectioned rat spinal cord model, MNTFs reduced inflammation, limited degeneration and enhanced regeneration of the grafted nerves (Wang et al., 1995). Furthermore, the wobbler mice with double recessive genes given one dose of 35 μg/kg MNTF1 at the age of six weeks slowed the neurodegenerative genetic disease in this strain.

Subsequently, the cloning of human MNTF1 and its associated receptor from a human retinoblastoma cDNA library was reported (Chau et al., 1993). Human MNTF1 cDNAs were subcloned into expression vectors and the MNTF1 polypeptides contained in the expressed fusion proteins exhibited biological activity similar to that of the "native" MNTF1 protein in that they supported the *in vitro* growth of rat anterior horn motoneurons. The amino acid sequences of the human MNTF1 polypeptides were elucidated by direct protein sequencing. One of the MNTF1 polypeptides consisting of 33 amino acids was identified as MNTF33mer, and subsequently was successfully synthesized by solid phase chemistry. The synthesized MNTF33mer showed biological activity in various *in vitro* and *in vivo* functional assays. A number of studies have demonstrated the trophic and tropic efficacy of the synthesized MNTF33mer in well-established rat peripheral nerve model systems. In a rat sciatic nerve transection with an 8-mm gap study, MNTF33mer treated animals have significant improvement of motoneuron regeneration in a dose response manner and promoted DRG neurons regeneration. In a transected femoral nerve rat model, the number of motoneurons projected correctly to muscle was enhanced in the MNTF33mer treated animals in a dose dependent manner. At the optimal dose, the number of motoneurons projected correctly to muscle was three times that of the motoneurons projected incorrectly to the skin.

In Silico Analysis was employed to search further for the active sites within the MNTF33mer molecule, and six active domain sites were identified. The smallest active site consists of 6 amino acids and hence was named MNTF6mer or GM6 (Chau, 2001). Recent studies have shown that GM6 had similar activity as the parent molecule (Chau, 2005, 2007). Studies with the synthesized GM6 also demonstrated similar trophic effects in a transected femoral nerve rat model. In a zebrafish bioassay, GM6 protected the organism from L-2-hydroxyglutaric acid (LGA) induced oxidative stress and apoptosis in the CNS, and reduced apoptosis by 85% in the midbrain. These studies suggest that MNTF33mer and its smaller analog GM6 provide protection from cell injury in a number of disease models.

Human MNTF1 is an endogenous neurotrophin, is highly specific for the human nervous system and it is expressed rapidly during the first trimester of human fetal development of the complete nervous system, peaking at week nine (Di and Huang, 1998). MNTF is a neuro-signaling molecule that binds on very specific receptors. The specific functions of MNTF, as demonstrated in animal and *in vitro* studies, include embryonic stem cell differentiation into motoneurons, motoneuron maintenance and survival, motor axon regeneration with guidance, and reinnervation of target muscles and organs (Chau et al., 1992). When the central nervous system (CNS) and peripheral nervous system (PNS) are under attack caused by diseases, disorders or injuries, MNTF creates a protective and permissive environment for nerve regeneration and repair that are neuroprotective, anti-apoptosis, anti-oxidation, anti-inflammation, and anti-scar.

Based on these data, we decided to test the ability of GM6 to protect the brain from acute ischemia and reperfusion injury.