NeuroToxicology 52 (2016) 46–56

Contents lists available at ScienceDirect

NeuroToxicology

Review

GDNF-induced cerebellar toxicity: A brief review

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ARTICLE INFO

Article history:
Received 1 July 2015
Received in revised form 22 September 2015
Accepted 24 October 2015
Available online 1 November 2015

Keywords:
GDNF
Parkinson's disease
Toxicology
Cerebellum
Purkinje cell
Putamen
Rhesus monkey
Convection-enhanced delivery

ABSTRACT

Recombinant-methionyl human glial cell line-derived neurotrophic factor (GDNF) is known for its neurorestorative and neuroprotective effects in rodent and primate models of Parkinson's disease (PD). When administered locally into the putamen of Parkinsonian subjects, early clinical studies showed its potential promise as a disease-modifying agent. However, the development of GDNF for the treatment of PD has been significantly clouded by findings of cerebellar toxicity after continuous intraputamenal high-dose administration in a 6-month treatment/3-month recovery toxicology study in rhesus monkeys. Specifically, multifocal cerebellar Purkinje cell loss affecting 1–21% of the cerebellar cortex was observed in 4 of 15 (26.7%; 95% confidence interval [CI]: 10.5–52.4%) animals treated at the highest dose level tested (3000 μg/month). No cerebellar toxicity was observed at lower doses (450 and 900 μg/month) in the same study, or at similar or higher doses (up to 10,000 μg/month) in subchronic or chronic toxicology studies testing intermittent intracerebroventricular administration. While seemingly associated with the use of GDNF, the pathogenesis of the cerebellar lesions has not been fully understood to date. This review integrates available information to evaluate potential pathogenic mechanisms and provide a consolidated assessment of the findings. While other explanations are considered, the existing evidence is most consistent with the hypothesis that leakage of GDNF into cerebrospinal fluid during chronic infusions into the putamen down-regulates GDNF receptors on Purkinje cells, and that subsequent acute withdrawal of GDNF generates the observed lesions. The implications of these findings for clinical studies with GDNF are discussed.

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http://dx.doi.org/10.1016/j.neuro.2015.10.011

1. Introduction

1.1. GDNF biology

Glial cell line-derived neurotrophic factor (GDNF), originally isolated from a rat glioma cell line in the early 1990s (Lin et al., 1993), is a distant member of the transforming growth factor-β superfamily and a founding member of the GDNF family of ligands (GFL), which includes neurturin, artemin and persephin (Airaksinen and Saarma, 2002). GDNF is a potent trophic factor for midbrain dopaminergic neurons, central noradrenergic neurons, spinal motor neurons and a variety of peripheral neurons. Outside the nervous system, it acts as a morphogen in kidney development and regulates spermatogonial differentiation (Airaksinen and Saarma, 2002).

Following intracellular processing, GDNF is secreted as a glycosylated mature protein of 134 amino acids (Lin et al., 1993). The active compound is a disulfide-bonded homodimer of approximately 30.4 kDa. As it lacks a specific carrier protein or transporter at endothelial cells, GDNF does not cross the blood–brain barrier (Kastin et al., 2003). In the adult human brain, GDNF is expressed at very low levels, with the highest concentrations in the caudate nucleus, putamen and substantia nigra (43–70 pg/mg protein), significantly lower concentrations in the cerebellum and frontal cortex (10–15 pg/mg protein), and undetectable concentrations (<8 pg/mL) in cerebrospinal fluid (CSF) (Mogi et al., 2001).

Cellular responses to all GDNF family ligands are mediated by a multicomponent receptor complex consisting of the membrane-anchored GDNF family ligand receptor (GFR)α and transmembrane RET receptor tyrosine kinase (Airaksinen and Saarma, 2002). Four different GFRα proteins (GFRα-1–4) with unique binding affinities for each ligand have been identified. GDNF preferentially binds to GFRα-1, but also interacts with GFRα-2 and GFRα-3, although at lower affinities (Airaksinen and Saarma, 2002). Effective GDNF signaling via GFRα-1-RET additionally requires the presence of heparan sulfate glycosaminoglycans which serve as high abundance, low-affinity receptors on the cell surface and in the extracellular matrix (Barnett et al., 2002; Tanaka et al., 2002). In cells lacking RET, especially in the forebrain, cortex and inner ear, the neural cell adhesion molecule (NCAM) has been identified as an alternate signaling receptor for GDNF, again requiring the co-expression of GFRα-1 for high affinity binding (Paratcha et al., 2003; Sariola and Saarma, 2003).

1.2. GDNF as a disease-modifying agent

Since its discovery, GDNF has received considerable attention as a drug candidate for the treatment of a variety of neurological diseases, most prominently Parkinson's disease (PD), with the understanding that the molecule needs to be delivered directly to the tissue of interest so as to bypass the blood–brain barrier to achieve meaningful tissue levels (Allen et al., 2013). In toxin-induced rodent and nonhuman primate models of PD, GDNF has been reproducibly shown to have both neurorestorative and neuroprotective effects and to improve motor function when delivered into the cerebral ventricles or directly into the dopamine-deficient striatum or substantia nigra (Tomac et al., 1995; Gash et al., 1996; Bjorklund et al., 1997; Zhang et al., 1997; Grondin et al., 2002).

Based on these promising animal data, GDNF was tested in four clinical studies enrolling a total of 99 subjects with PD. In the first study, monthly intracerebroventricular (ICV) bolus injections of GDNF failed to provide clinical benefit relative to placebo and were associated with a number of gastrointestinal side effects including nausea, anorexia and vomiting and induced both weight loss and hyponatremia in over half of the subjects (Nutt et al., 2003). A postmortem analysis in one of the GDNF-treated study subjects showed no GDNF immunoreactivity and no appreciable increase in putamenal tyrosine hydroxylase immunoreactivity relative to age-matched control cases with PD (Kordower et al., 1999). Therefore, ICV delivery was subsequently replaced with intraputamenal (IPu) delivery using implantable pumps. In addition, intermittent bolus administration was replaced with continuous administration by infusion, as the pumps required minimum basal infusion rates to maintain proper function (Gash et al., 2005).

With these changes, GDNF showed strong signs of efficacy in two uncontrolled open-label Phase I studies (Gill et al., 2003; Slevin et al., 2005), but was not significantly different from placebo in a randomized placebo-controlled Phase II study (Lang et al., 2006). In contrast to the ICV study, GDNF was found to be well tolerated and clinically safe in all of the IPu studies (Gill et al., 2003; Slevin et al., 2005; Lang et al., 2006), although more than half of the subjects treated with GDNF developed clinically asymptomatic immune responses with binding antibodies to the protein, including 5 subjects with neutralizing antibodies (Tatarewicz et al., 2007).

1.3. GDNF pharmacokinetics

Both in mammalian cell cultures and in vivo, GDNF undergoes N-terminal proteolytic cleavage of 31–37 amino acid-long fragments including the main heparin-binding site of the molecule (Lau, 1996). Truncated GDNF remains biologically active in soluble form, although not in immobilized matrix-bound form which requires interaction with the heparan sulfate chains of syndecan-3, a transmembrane proteoglycan receptor, for proper signal transduction (Bespalov et al., 2011).

Plasma concentrations in normal rhesus monkeys after single ICV infusions of exogenous GDNF (recombinant-methionyl human GDNF, r-metHuGDNF) at different doses (100 and 500 μg) were detectable only sporadically within the first hour post dosing, the highest individual value being 2.11 ng/mL (Lau, 1996). By contrast, mean peak GDNF concentrations in CSF (C_{CSF}) were 3554 ng/mL (100 μg dose) and 33,975 ng/mL (500 μg dose), respectively (Lau, 1996). The terminal half-life in CSF of GDNF after these doses was found to be assay-dependent. With an assay detecting only full-length GDNF, the terminal half-life was 34 h, while it was almost threefold longer (92 h) with an assay capturing both full-length and truncated GDNF, indicating that in vivo processing of GDNF occurs in CSF (Lau, 1996). The pharmacokinetics of GDNF in CSF of normal rhesus monkeys were further found to hinge on the proximity of the sampling site (lumbar spine or cisterna magna) to the site of administration (intrathecal lumbar or ICV). After administration of the same single doses, higher peak concentrations, larger areas under the curve and longer half-lives were found at the sampling site that was closer to the site of administration than at the more distant sampling site (Wong, 2003a,b). Binding of GDNF to local heparan sulfate glycosaminoglycans is considered