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Short communication

Differential mRNA expression of neurotrophic factors GDNF, BDNF, and NT-3 in experimental herpes simplex virus encephalitis

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Abstract

Glial cell line-derived neurotrophic factor (GDNF), brain-derived neurotrophic factor (BDNF), and neurotrophin-3 (NT-3) mRNA levels were studied in the course of murine herpes simplex virus encephalitis. Induction of GNDF and NT-3 (both $P < 0.05$) was found during acute encephalitis. Despite absence of clinical impairment, both neurotrophic factors were overexpressed 2 months (NT-3) and 6 months (GDNF) following infection (both $P < 0.05$). Neurotrophic factors play an important role in neuronal survival and recovery after acute injury to the central nervous system (CNS) and may represent an additional therapeutic target for treatment of viral encephalitis.

Theme: Development and regeneration
Topic: Neurotrophic factors: expression and regulation

Keywords: Viral encephalitis; Herpes simplex virus; Neuroregeneration; Brain injury

Herpes simplex virus 1 is the most common cause of non-epidemic focal encephalitis (herpes simplex virus encephalitis; HSVE) [16]. However, over 70% of the survivors suffer from significant morbidity in the long term [23], indicating the utmost importance for a better understanding of pathophysiological mechanisms of axonal damage and regeneration in HSVE.

Recently, studies reported on the expression of the neurotrophic factors glial cell line-derived neurotrophic factor (GDNF), brain-derived neurotrophic factor (BDNF), and neurotrophin-3 (NT-3) after different types of brain injury and activation through pro-inflammatory mediators. GDNF is a member of the transforming growth factor-beta (TGF-b) superfamily and plays an important role in survival and recovery of mature neurons [2,5,31]. Promising data were shown in experimental models of cerebral ischemia after treatment with GDNF, where subsequent reduction of ischemic damage and promotion of axonal and glial regeneration could be achieved [17,19,35]. BDNF is a neurotrophin known for participation in neuronal survival after cerebral ischemia [18] and is capable of reducing multiple forms of brain injury in bacterial meningitis [4]. In contrast, NT-3 has been demonstrated to promote cell death induced by cerebral ischemia, oxygen-glucose deprivation, and oxidative stress [3].

We addressed the question whether these factors are involved in the pathogenesis of HSVE by studying the mRNA expression of GDNF, BDNF, and NT-3 in whole brain tissue during acute (day 3 and 7 post inoculation, d.p.i.) and remote phase (2 and 6 months p.i.) of experimental murine HSVE. A subgroup of animals was treated with acyclovir or acyclovir plus methylprednisolone (MP) with regard to recent reports on favorable effects of adjunctive cortisone in viral encephalitis [27,30].

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All experiments were performed according to the guidelines provided by the European Communities Council (86/609/EEC) and were approved by the National Ethics Committee for Animal Research. We used a previously described animal model utilizing the neurovirulent wild-type HSV-1 strain F in order to produce a focal encephalitis resembling human disease [11,25]. Briefly, female SJL mice (Bolmholtgard Breeding and Research center, DK) were anesthetized with ketamine and inoculated intranasally with 20 μl (equivalent to 10^5 plaque-forming units) virus suspension. Sham-infected control animals received the same amount of sterile water. Animals were randomly assigned to three treatment groups (treatment over 14 days with intraperitoneal injection two times per day): group 0: saline; group 1: acyclovir (GlaxoSmithKline, Munich, Germany) 50 mg/kg/day; group 2: acyclovir and MP (Aventis Pharma, Bad Soden, Germany) 40 mg/kg/day. Clinical scoring was performed daily and animals were sacrificed by a lethal dose of ketamine/pentobarbital at 3 and 7 days (d.p.i.), and 2 and 6 months post inoculation (m.p.i.), respectively. Brains were quickly removed and stored at −80 °C until processed. Brain tissue was homogenized in ATL-buffer (Qiagen, Hilden, Germany), and DNA was isolated with DNAzol (Invitrogen, Karlsruhe, Germany) and total RNA with RNAClean (Amersham Biosciences, Germany) according to the manufacturer's instructions, respectively.

Quantitation of viral copies from brain tissue was performed exemplary in sham-infected (n = 4) and in infected animals at 7 d.p.i. (n = 4 each; groups 0, 1, and 2) and 2 m.p.i. (n = 3; group 0) as previously described [24,36]. Briefly, a nested PCR was performed from DNA with two sets of primers specific for the glycoprotein D gene of HSV-1 in an Eppendorf Mastercycler (Eppendorf, Hamburg, Germany).

Semiquantitative real-time reverse transcription polymerase chain reaction of GDNF, BDNF, and NT-3 was performed with a SYBR-Green real-time quantitative PCR kit in an ABI PRISM 5700 Sequence Detector and primers were designed with Primer Express Software Version 2.0 by homology search (all: Applied Biosystems, Weiterstadt, Germany). Before first-strand cDNA was synthesized by using avian myeloblastoma virus reverse transcriptase and oligonucleotide dT primers (Promega, Madison, Wisc.), potential trace amounts of residual DNA were removed by digestion with RNAse-free DNAse (Quiagen, Hilden, Germany). A standard curve was calculated by measuring serially diluted amounts of cDNA and mRNA levels were calculated as % of the housekeeping gene glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (relative units, ru). The sequence of GAPDH has been reported previously [24] and the characteristics of the other primers are described in Table 1. The amplification reactions were performed in duplicate with 200 ng cDNA and contained 1.25 U AmpliTaq Gold, SYBR-Green Mix, 250 μM dNTPs, and 300 nmol sense/antisense primers in a total reaction volume of 30 μl. Negative controls were included in each reaction. The temperature profile consisted of one initial cycle at 50 °C for 2 min and at 95 °C for 10 min, and were followed by 40 reactions at 95 °C (15 s) and 60 °C (60 s). Following the amplification, a dissociation curve was acquired by applying increasing temperature. The absence of non-specific amplification was checked by analysis of the dissociation curve and the identity of the product was verified by sequencing after subcloning into the vector pCRII-TOPO (Invitrogen, Groningen, Netherlands). Gene expression levels during the course of HSVE were studied by one-way ANOVA with Bonferroni's post hoc test and significance was set at $P < 0.05$. Comparison of gene regulation between different treatment groups was performed with Student's t test.

The earliest clinical signs in infected animals were evident at day two and peaked at day seven to nine in all animals infected with HSV-1. Clinical impairment of treated and untreated animals did not differ significantly and no disability was evident from 21 d.p.i., as reported recently [27]. At 7 d.p.i., mean viral load of infected, saline-treated animals (group 0) was 191.4 ± 280.4 virus copies (vc)/μg DNA and almost returned to baseline (22.1 ± 20.0 vc/μg DNA) at 2 m.p.i. In brains from animals of group 1, we detected 113 ± 82 vc/μg DNA, and in group 2, 119 ± 97 vc/μg DNA at 7 d.p.i. Sham-infected animals had neither changes of their clinical score nor presence of viral copies within their brains.

GDNF levels (Fig. 1a) in sham-infected mice were detected at the baseline level of 1.35 ± 0.93 relative units (ru; mean ± SD). Inoculation with HSV-1 and saline treatment (group 0) led to an increase of GDNF expression at 3 d.p.i. (4.01 ru ± 4.43) and peaked at 7 d.p.i. (6.85 ru ± 4.68; $P < 0.05$ compared to sham-infected mice). At 2 m.p.i., the expression of GDNF returned to baseline levels

Table 1
List of primer characteristics

Gene	Accession no.		Sequence (5′→3′)	Amplicon size (bp)
GDNF	D883519	Sense	AGC ACT TCA GGG TGG CAG C	51
		Antisense	AGG ACA CCA GCC CTG AGC	
BDNF	NM007540	Sense	GCA CTG GAA CTC GCA ATG C	50
		Antisense	GTA AGG GCC CGA ACA TAC GA	
NT-3	NM008742	Sense	AGC CAA TGA TTG CAA CGG A	52
		Antisense	TTG TAG CGT CTC TGT TGC CG	