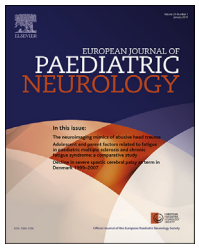
Official Journal of the European Paediatric Neurology Society



Original article

CSF nerve growth factor (β-NGF) is increased but CSF insulin-like growth factor-(IGF-1) is normal in children with tuberous sclerosis and infantile spasms

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ARTICLE INFO

Article history:
Received 25 February 2018
Received in revised form
1 November 2018
Accepted 1 November 2018

Keywords:
Tuberous sclerosis
Nerve growth factor
Insulin-like growth factor
Infantile spasms

ABSTRACT

Tuberous sclerosis is associated with epilepsy that is often refractory. We examined cerebrospinal fluid (CSF) concentrations for neurotrophins, nerve growth factor (β-NGF) and insulin-like growth factor (IGF-1) in children with infantile spasms between 1997 and 2010. We classified the patients as follows: tuberous sclerosis (n = 5), cryptogenic spasms (n = 6), postinfectious spasms (n = 5) and other symptomatic spasms (n = 22). We had 22 age- and sex-matched controls for CSF-NGF and 14 for CSF-IGF-1. The median of CSF-NGF was higher in those with tuberous sclerosis, 56 (minimum–maximum, 8.0–131) ng/L, in relative to age- and sex-matched controls, 6.7 (0.0–22) ng/L, and symptomatic infantile spasms, 0.0 (0.0–4.5) ng/L or cryptogenic cases of infantile spasms, 6.2 (3.9–8.8) ng/L, respectively. CSF-NGF were highest in children with postinfectious aetiology, 408 (89–778) ng/L. CSF-IGF-1 of tuberous sclerosis, 0.65 (0.35–0.98) μg/L, did not differ from the cryptogenic spasms, 0.68 (0.32–0.87) μg/L, or from age- and sex-matched controls 0.52 (0.22–0.77) μg/L. Patients with tuberous sclerosis and cryptogenic spasms had normal development prior the ACTH therapy. We suggest that increased CSF-NGF might indicate a persistent activation of inflammatory pathways in cortical tubers in tuberous sclerosis and this would reflect in CSF concentrations.

1. Introduction

Mutations of tuberous sclerosis complex-1 (TSC1) and TSC2 tumour suppressor genes result in hyperactivation of the mammalian target of rapamycin (mTor) signalling pathway and subsequent abnormalities in numerous cell processes. Neurotrophins and their cognate receptors compose a family of proteins that mediate proliferation, migration, and growth and refinement of neural connections during cortical development. Information is rare in human tissue containing either nerve growth factor (NGF) or insulin-like growth factor-1

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https://doi.org/10.1016/j.ejpn.2018.11.001
1090-3798/

(IGF-1) in patients with tuberous sclerosis (TS). Neurotrophins are expressed throughout cortical development modulating cortical development. It has been proposed that neurotrophins could be ideal candidate molecules to investigate in tubers.[1]

NGF synthesis is influenced by hormones and the immune system.[2] Inflammatory mechanisms may contribute to the neurological manifestations of TS complex.[3–5] mTor-mediated inflammatory mechanisms may be involved in epileptogenesis in TS.[5] There are persistent activation of inflammatory pathways in cortical tubers and subependymal giant cell tumours of patients with TS-complex.[4] NGF may be involved in inflammatory process and hypersensitivity reactions.[4] NGF acts as a pleiotropic cytokine at the interface between the neurons and immune system.

IGF-1 is a key factor that regulates neurogenesis and synaptogenesis from embryonic to adult stages of the brain.[6,7] IGF-1 acts on mTor. Tuberins and hamartomas have a genetic link to IGF-1 pathway.[8] IGF stimulates phosphorylation of tuberin. Phosphorylation may be a major mechanism of regulation of the hamartin-tuberin complex.

Everolimus is a selective mTor antagonist. Recently it has been shown to control seizures in patients with TS and it may have beneficial effects on behaviour and quality of life.[9–12]

The aim of the present study was to measure cerebrospinal fluid (CSF) neurotrophins of patients with TS and infantile spasms (IS) hypothesising that CSF-IGF-1 or -NGF might reflect persisting activation of inflammatory pathways in cortical tubers and have impact on the pathophysiological mechanisms of TS.

2. Material and methods

The study protocol was approved by the Ethical Committee of Helsinki University Hospital, Helsinki, Finland and had institutional approval. Informed consent for examinations was obtained from each patient's parents. We enrol the subjects between 1997 and 2010.

We investigated patients with IS for CSF-NGF (n = 38) and for CSF-IGF-1 (n = 26). These studies included five patients with TS for CSF-NGF and five patients for CSF-IGF-1. We had 22 age- and sex-matched controls for CSF-NGF and 14 age- and sex-matched controls for CSF-IGF-1. Because it would have been unethical to draw CSF from healthy children we had controls with some slight neurological system disorder. The controls for CSF-NGF were age- and sex-matched patients with neurological disease. In these children CSF was drawn to exclude a central nervous system infection or reveal other aetiology of the disorder. No control patients had an acute infection, epilepsy, or mental retardation, which might possibly have influenced the concentration. Control group for CSF-IGF-1 concentrations served a cohort of patients with some neurological diseases other than epilepsy. None of the controls had diabetes, current infection, hyperthyroidism, liver disease, or oncologic problems, conditions that might have influenced the CSF-IGF-1 concentration. The early history of the controls had been uneventful in most cases.

The patients were admitted to Children's Hospital, University of Helsinki for diagnosis and treatment. The neurotrophin studies were made at the time of evaluation for aetiology of IS.[13,14] All patients were carefully examined to exclude acute infections before start of adrenocorticotropic hormone (ACTH) -therapy.

The NGF concentration was determined by a sensitive two-site enzyme-linked immunosorbent assay. Human NGF was used as standard (gift from Genentech Inc., San Francisco, CA, USA). IGF-1 was determined by radioimmunoassay with a commercially available kit (Medidiagnost, Tuebingen, Germany) according the manufacturer's instructions.

Data are expressed as median [minimum–maximum] as appropriate. CSF-NGF and -IGF-1 concentrations between controls and children with IS were analysed by the Kruskall-Wallis-test and for pairwise comparison we used Mann–Whitney U-test. For multiple comparisons, the Bonferroni correction was applied. Categorical data were analysed using the Chi-Square-test. These analyses were made by means of the Statistical Package for Social Science-software (SPSS 22.0, IBM Corporation, Armonk, NY, USA).

3. Results

The main results are shown in Table 1. The aetiology was classified as cryptogenic in six patients. The aetiology of symptomatic IS (n = 22) (excluding in this number TS (n = 5) and postinfectious spasms (n = 5)) were prenatal (n = 5), postnatal (n = 2), asphyxia (n = 3), brain infarct (n = 1), malformations of central nervous system (n = 4), Down syndrome (n = 3), other chromosomal abnormality (n = 1), progressive encephalopathy with oedema, hypsarrhythmia and optic atrophy (PEHO) -syndrome (n = 1), and metabolic disorder (n = 2).

The median of age in patients with IS (14 girls/24 boys) for CSF-NGF measurement was 7 [minimum–maximum, 2.5–20] months and that in the controls (12 girls/10 boys) 12 [1–24] months (sex, p = 0.28; age, p = 0.081). The median of age in patients with IS (12 girls/10 boys) for CSF-IGF-1 measurement 6.5 [2–16] months and that in the controls (10 girls/4 boys) 9.5 [3–26] months (sex, p = 0.105; age, p = 0.171).

3.1. *NGF in infantile spasms*

The median CSF-NGF concentration of patients with TS (n = 5) was 59 [8.0–131] ng/L and that of symptomatic (excluding TS and postinfectious groups) patients 0.0 [0.0–4.6] ng/L, i.e. patients with TS had 100-times higher concentrations of CSF-NGF compared to those with symptomatic aetiology (n = 22).

Table 1 – Cerebrospinal fluid (CSF) nerve growth factor (NGF) and insulin-like growth factor-(IGF-1) in controls and in children with tuberous sclerosis and infantile spasms. Data are median [minimum–maximum].

Variable	CSF-NGF ng/L	CSF-IGF-1 μg/L
Controls	6.7 [0.0–22], n = 22	0.52 [0.22–0.77], n = 14
Tuberous sclerosis	56 [8.0–131], n = 5	0.65 [0.35–0.98], n = 5
Cryptogenic spasms	6.2 [3.9–8.8], n = 6	0.68 [0.31–0.87], n = 6
Symptomatic infantile spasms	0.0 [0.0–4.6], n = 22	0.21 [0.16–0.91], n = 15
Postinfectious aetiology	408 [89–778], n = 5	na

Before ACTH-therapy, infants with cryptogenic aetiology had normal concentrations of CSF-NGF, 6.2 [3.9–8.8] ng/L. All infants with post-infectious aetiology (n = 5) had very high CSF-NGF concentration, 408 [89–778] ng/L.

The median CSF-NGF concentration of patients with cryptogenic aetiology did not differ from that in the controls with 6.7 [0.0–22] ng/L. The concentrations of the symptomatic group (excluding TS and post-infectious-groups) differed from the cryptogenic patients ($P < 0.001$) and from the controls ($P < 0.001$). Low/lacking NGF might reflect massive neuronal death in patients seen as atrophy in computed tomography. CSF concentrations of the postinfectious-group were higher than the concentrations in children with cryptogenic aetiology ($P = 0.004$). IS occurred 1.5–2.5 months after encephalitis (ECHO22 virus (n = 1), herpes simplex (n = 2), adenovirus (n = 1) and unknown virus (n = 1)). There was no evidence blood-brain-barrier damage in these children; CSF-protein (ranging between 290 and 500 mg/L) and CSF-albumin (99–230 mg/L), and serum-/CSF-albumin -ratios, (measured in 3 of the 5 infants, 199, 356 and 429, respectively) were all within normal limits. See Fig. 1.

3.2. *IGF in infantile spasms*

Patients with TS had the median CSF-IGF-1 concentration, 0.65 [0, 35–0.98] μg/L, similar to cryptogenic IS, 0.68 [0.31–0.87] μg/L, p = 0.54.

Infants with cryptogenic aetiology had CSF-IGF-1 concentrations similar to controls, 0.52 [0.22–0.77] μg/L, but children with symptomatic IS had markedly low concentrations, 0.21 [0.16–0.91] μg/L. See Fig. 2.

4. Discussion

We have found high CSF-NGF concentrations in TS patients compared to other symptomatic IS patients. Of note, however, a separate group of postinfectious aetiology patients had exceptionally high NGF production. The reason for high NGF production is not clear but might be a prolonged hyperactivity of neurons both in patients with TS, and with IS of post-infectious aetiology.[13,15,16]

Tuberous sclerosis is a genetic disorder characterized by the development of tumour-like lesions (hamartomas). These tubers are cortical developmental malformations and are composed histologically distinct cell types, including giant cells and dysplastic neurons. These embryonic tubers are highly epileptogenic.[17] Tuberous sclerosis affects tissues from different germ layers. Hamartin and tuberin function is a complex that interacts with RHEB "Ras homolog enriched in brain". It is a GTP-binding protein (a proximal activator of mTor1) GTPase thus sequestering it from activating mTor signalling, part of the growth factor (insulin) pathway.[8] Thus,

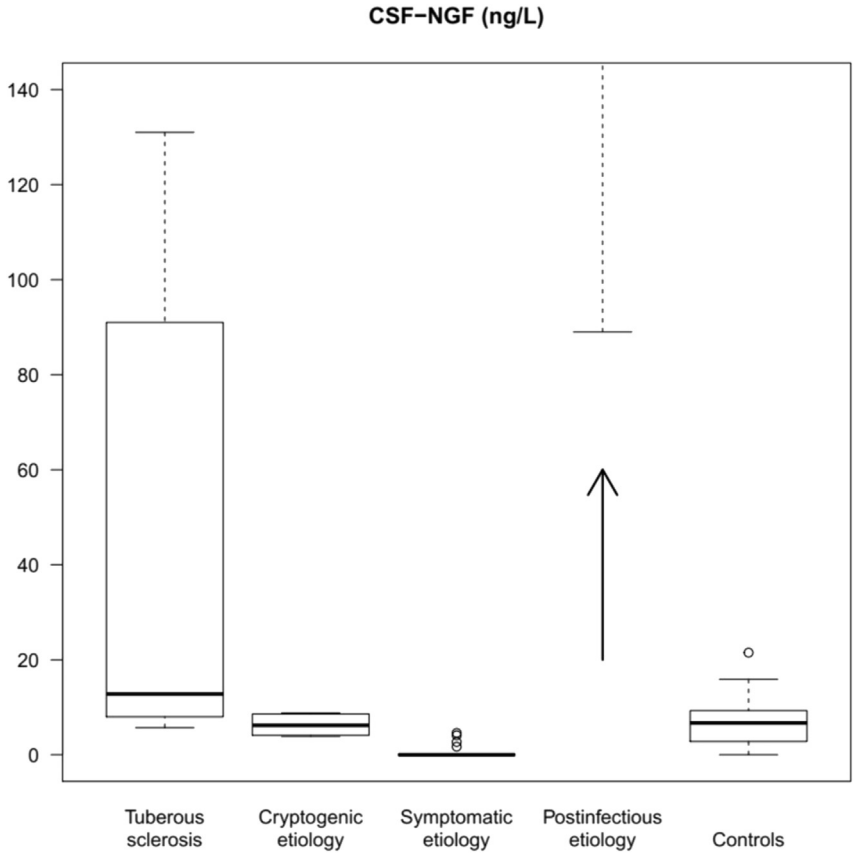

Fig. 1 – Cerebrospinal fluid (CSF) nerve growth factor (NGF) in controls (n = 22), in children with tuberous sclerosis (n = 5) and in children with infantile spasms of cryptogenic (n = 6), symptomatic (n = 22) and postinfectious aetiology (n = 5). Infants with post-infectious aetiology had CSF-NGF concentration between 89 and 778 (median 408) ng/L.

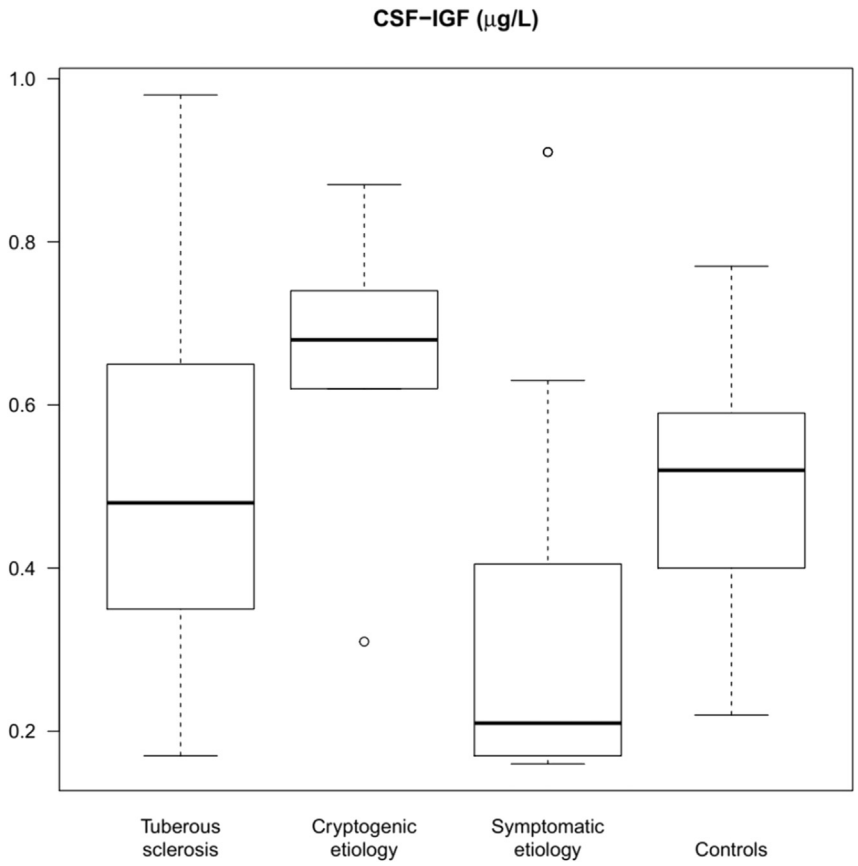

Fig. 2 – Cerebrospinal fluid (CSF) insulin-like growth factor-(IGF-1) in controls (n = 14), in children with tuberous sclerosis (n = 5) and in children with infantile spasms of cryptogenic (n = 6) and symptomatic aetiology (n = 15).

mutations at the TSC1 and TSC2 loci result in a loss of control of cell growth and cell division, and therefore a predisposition to forming tumours.

4.1. *IGF-1*

CSF-IGF-1 of TS did not differ from the cryptogenic spasms, or from age- and sex-matched controls. Because it would have been unethical to draw CSF from healthy children we had controls with slight neurological diseases.

In another study we had 16 healthy children who were admitted to Kuopio University Central Hospital for surgery on the lower part of the body (e.g., inguinal hernia), to be performed under spinal anesthesia.[13] The healthy children with minor surgery had similar CSF-IGF-1 concentrations as the control children of this study.[18] Our five patients with TS had a good early development before the spasms onset, as well in our earlier series most of TS and cryptogenic patients had good cognition before IS and good response to ACTH-therapy (73% and 80%), respectively.[19] In another earlier study patients with IS with high CSF IGF-1 concentrations before the ACTH-therapy had better later cognitive outcome than those with low concentrations.[20]

Both synaptic connectivity and neuro-inflammatory processes may involve the P13K-Akt-mTor pathway. IGF-1 is important for synaptic connectivity.[6] Effects of IGF-1 may be mediated by modulation of P13-Akt-mTor pathway.[21]

4.2. *NGF*

The significance of over-stimulation of CSF-NGF production in our patients is still far from clear. We do not know if it is a protective mechanism for survival of neurons, or a sign of uncontrolled signalling of tyrosine kinase A (Trk A) -receptors which must be stopped.

It is proposed that by blocking NGF or mediators released upon NGF activation, the progression of inflammation could be controlled; thereby opening therapeutic opportunity for the future.[22] CSF-NGF would appear to be a feasible biomarker of immune mechanisms functioning in central nervous system.

4.3. *Rapamycin*

Activation of rapamycin sensitive mTor signalling mediates NGF induced cell migration. Rapamycin, the pharmacologic inhibitor of mTor), diminished NGF induced phosphorylation and cell migration.[22] Blockade of inflammation may prove to be a significant benefit in inflammatory status. m-Tor-mediated inflammatory mechanisms may be involved in epileptogenesis in TS.[5] It is possible that seizure activity triggers microglial activation observed within dysplastic cortex but

deserves further investigation.[4] In animal experiments short-term mTor inhibition in TS by rapamycin has been demonstrated to normalize development and eliminate seizures even with short term treatment early in life.[23] In children significant seizure reduction has been shown,[9] but he results on improvement of neurocognitive functions are mixed.[24]

4.4. *Protein kinase inhibitors*

NGF signals through Trk A -receptors. Tyrosine kinases are involved in embryonic development, metabolism, cell proliferation, and immune system leading to enhanced and sometimes persistent stimulation – to proliferative diseases, including cancers, psoriasis, and others. A promising targets for drug design for some proliferative diseases may be protein kinase inhibitors.[25–28]

Other agents blocking inflammation and decreasing seizures in TS in animals have been anti-inflammatory agents.[5] Steroids (ACTH) in the treatment of IS in TS have shown an excellent response on spasms.[19]

Furthermore, Mills et al.[29] recently observed increased expression of genes in TS associated with inflammatory, innate and adaptive immune responses. In contrast, down-regulation of genes associated with neurogenesis and glutamate signalling. That study provided new insights into the TS transcriptomic network along with the identification of potential new treatment targets.

The main limitation of our study is small patient number. Furthermore, it remains to be seen whether there is a difference in CSF biomarker concentrations in TS with or without IS or other forms of epilepsy, to find out whether the CSF-NGF concentration might be a biomarker of the epilepsy itself. However, such a study might be difficult but not impossible to perform because of the relative infrequency with which CSF specimens are obtained. It would reveal whether NGFs are really involved in pathophysiological mechanisms of TS, or sign of epileptic activity.

5. Conclusion

Our findings indicate that increased CSF-NGF might be a feasible biomarker of immune mechanisms functioning within the cortical tubers in TS. It might be involved in pathophysiological mechanisms, and thus opening therapeutic opportunity.

Conflict of interest

Riikonen & Kokki: CSF nerve growth factor (β-NGF) is increased but CSF insulin-like growth factor (IGF-1) is normal in children with tuberous sclerosis and infantile spasms.

We declare that we have no conflict of interest to report.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejpn.2018.11.001.

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