The Effects of Vagus Nerve Stimulation on Pro- and Anti-Inflammatory Cytokines in Children with Refractory Epilepsy: An Exploratory Study

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Key Words

Vagus nerve stimulation · Epilepsy · Children · Inflammation · Cytokines · Cholinergic anti-inflammatory pathway

Abstract

Objectives: The vagus nerve has important immunological and anti-inflammatory actions that might be relevant to the beneficial effects of vagus nerve stimulation (VNS). Therefore, we conducted an exploratory study on VNS effects on cytokine levels in plasma and cerebrospinal fluid of children suffering from refractory epilepsy. Moreover, as predictors of the response are lacking, we also aimed to determine if cytokine changes predict the clinical response. **Methods:** VNS was performed according to a randomized double-blind design: plasma levels were compared between patients who received 20 weeks of high output (therapeutic) ($n = 21$) or low output (active control) stimulation ($n = 20$). Thereupon, all patients received high output stimulation for another 19 weeks; levels during this period were compared to baseline. Interictal interleukin-1 β , interleukin-6, and interleukin-10 were determined by ELISA. **Results:** No significant changes were found between high and low output groups and between the last 19 weeks of stimulation and baseline. Chang-

es in interleukin-1 β correlated with improved IQ ($\tau = 0.42$, $p < 0.01$). Lower baseline plasma levels of interleukin-6 were associated with more seizure frequency reduction [$R^2 = 0.105$ (1, 35), $p = 0.050$]. **Conclusion:** Interictal cytokine levels were not altered by VNS but baseline interleukin-6 predicted the clinical response. In the future, patient selection may be aided by determination of the cytokine profile of the patient.

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Introduction

Vagus nerve stimulation (VNS) is an adjunctive treatment for medically refractory epilepsy. In addition to anticonvulsive effects, VNS has positive effects on behavior, mood, and cognition [1]. Not all patients respond to VNS: in epilepsy, 23–39% of VNS-treated patients experience more than 50% seizure frequency reduction versus 13–19% of placebo-treated patients who received nontherapeutic low output stimulation [2–4]. It is unclear why the effects of VNS are limited and predictors to identify patients that are most likely to respond are lacking. Moreover, the mechanism of action of VNS is not fully understood.

Table 1. Patient characteristics

	High output VNS	Low output VNS
Number of patients (male/female)	21 (11/10)	20 (12/8)
Mean age at implantation (range)	10y 11m (3y 10m to 17y 8m)	11y 6m (4y 2m to 17y 2m)
Mean age at onset (range)	2y 10m (0–12)	1y 8m (0–5)
Mean interval onset-implantation (range)	7y 8m (2–16)	9y 5m (3–15)
Median seizure frequency at baseline (range), seizures/day	2.1 (0.1–53.7)	0.9 (0.1–31.7)
ILAE classification [41]		
Localization related	19 (90%)	16 (80%)
Symptomatic	15 (71%)	10 (50%)
Cryptogenic	4 (19%)	6 (30%)
Generalized	2 (10%)	4 (20%)
Idiopathic	0	2 (10%)
Symptomatic	2 (10%)	2 (10%)
Total number of AEDs ever administered (range)	7.0 (5–10)	7.3 (4–14)
Mean number of AEDs used during the study (range)	2.5 (1–5)	2.5 (1–4)
Mean maximal output, mA		
During blinded phase	0.99	n.a.
During add-on phase	1.98	1.29

y = Years; m = months; n.a. = not applicable.

In this context, the immunomodulatory function of the vagus nerve is of particular interest. Afferent signals can activate the so-called cholinergic anti-inflammatory pathway upon inflammation [5]. Through this pathway, efferent vagus nerve fibers inhibit the release of pro-inflammatory cytokines and in this way reduce inflammation. In recent years, inflammation has been strongly implicated in the development of seizures and epilepsy [6] and therefore the activation of the anti-inflammatory pathway by VNS could decrease the inflammatory response and thereby explain its clinical effects.

While several animal studies have demonstrated that electrical and chemical stimulation of the vagus nerve affects the immune response [7–16], only a few studies on the immunomodulatory effects of VNS have been performed in humans. VNS has been reported to increase circulating pro- and anti-inflammatory cytokines in depressed patients [17], but in epilepsy patients the results are inconclusive: VNS has been shown to alter the immune response [18] but fails to affect systemic inflammation [19, 20]. So far there are no studies available evaluating neuro-immunomodulation by VNS in children. Therefore, the purpose of this study was to determine whether VNS treatment caused changes in cytokine levels in children and if these changes were related to such clinical effects as seizure frequency reduction and IQ. Furthermore, we aimed to determine if the changes in

cytokine levels could predict the clinical response. Therefore, we determined levels of interleukin (IL)-1 β and IL-6, while these cytokines were increased in epilepsy patients [21–26]. Additionally, we also evaluated levels of IL-10, as this is one of the most important anti-inflammatory cytokines [27].

Patients and Methods

Patients

We included 41 children (23 boys) with medically refractory epilepsy, which was defined as failure of adequate trials of at least two tolerated, appropriately chosen and used antiepileptic drug (AED) schedules to achieve sustained seizure freedom [28]. The mean age at implantation was 11.2 years. Thirty-five patients had localization related epilepsy, and 6 patients had generalized epilepsy (for details see table 1). Twenty patients had focal seizures, 11 patients had generalized seizures, and 10 patients had both focal and generalized seizures. All patients had undergone an extensive clinical evaluation, which included EEG, CT/MRI, and genetic and/or metabolic screening as needed. Patients with a progressive cerebral lesion, malignancy, or any degenerative or immunological disorder such as Rasmussen encephalitis or acute disseminated encephalomyelitis were excluded. Medication regimens remained unchanged throughout the study and none of the patients were treated with antihistamines, metoclopramide, or anti-inflammatory drugs. Visits were postponed if patients displayed any clinical signs of infection during the study. The most frequent administered AEDs included valproic acid (n = 18), la-

Fig. 1. Study design. After a 12-week baseline, patients were implanted with the vagus nerve stimulator. During the double-blind phase (20 weeks), half of the patients received high output stimulation and the other half received low output stimulation. During the add-on phase (19 weeks), all patients received high stimulation. Time is presented in weeks. Arrows indicate data and sample collection. B = Blood.

Table 2. Initial device settings

	Active control group	Treatment group
Output current, mA	0.25	0.25
Pulse width, ms	0.1	0.5
Frequency, Hz	1	30
Duty cycle: on (s)/off (min)	14/60	30/5

motrigine (n = 16), clobazam (n = 11), topiramate (n = 10), and levetiracetam (n = 9).

After a baseline period of 12 weeks, patients were implanted with a programmable pulse generator (Neurocybernetic prosthesis NCP; Cyberonics Inc., Webster, Tex., USA). Patients were randomly assigned to two groups: patients who received either high (therapeutic) or low (active control) stimulation. This active control group was incorporated to protect the blinding, because patients can notice stimulation, and to control for the presence of the electrode. Initial stimulation parameters are depicted in table 2. In the treatment group, current was stepwise increased with 2-week intervals to the maximally tolerated output current (maximum 1.75 mA). After 20 weeks, all patients entered an unblinded follow-up of 19 weeks. During this add-on phase both groups received high output stimulation (maximum 2.25 mA) (see fig. 1 for study design).

All procedures were approved by the Central Medical Ethical Committee of Maastricht University Medical Centre and written informed consent was obtained from all caregivers and patients if aged over 12 years.

VNS Effects

We evaluated VNS effects on seizure frequency and cognitive function. Seizure frequency was recorded in a seizure diary by caregivers. The effect of VNS was calculated by expressing the mean seizure frequency during the last 19 weeks of VNS as a percentage of the seizure frequency at baseline.

Cognitive function was assessed using the Peabody picture vocabulary test (PPVT-III-NL) [29] and the Beery VMI 5th edition [30]. To calculate IQ, raw scores were converted to age-equivalents in months, which were divided by the age in months at the date of testing and multiplied by 100. Finally, the average of both tests was calculated.

Cytokine Measurements

EDTA plasma was obtained at baseline, at the end of the blinded phase after 20 weeks of stimulation, and at the end of the add-on phase after 39 weeks of stimulation (fig. 1). Plasma and cerebrospinal fluid (CSF) were also collected immediately before surgery when patients were under general anesthesia. Patients did not experience seizures in more than 12 h preceding sample collection in order to determine interictal, overall cytokine levels.

Levels of IL-1 β , IL-6, and IL-10 were determined in duplicate using commercially available enzyme-linked immunosorbent assays (R&D Systems, Abingdon, UK; detection limits 0.125, 0.156, and 0.78 pg/ml, respectively) following the manufacturer's instructions. Values below the detection limit were converted to a value of 0.5 times the detection limit for statistical analysis, as these cannot be considered missing [20, 31].

Statistics

Statistical analysis was performed using SPSSv19 for MacOSX. Cytokine levels at the end of the blinded phase were compared between the high and low output groups using the Mann-Whitney U test. Cytokine levels at the end of the add-on phase were compared with baseline by a related-samples Wilcoxon signed-rank test.

To analyze the relationship between cytokine changes and VNS effects, we performed a correlation analysis (Kendall's tau) between the percentage change of cytokines and IQ and seizure frequency.

To evaluate whether baseline and pre-operative cytokine plasma and CSF levels could predict seizure frequency reduction, univariate linear regression analysis was performed using seizure reduction during the add-on phase as the dependent variable. $p < 0.05$ was considered statistically significant.

Results

There were no statistically significant differences in cytokine levels between the high and low stimulation groups at the end of the blinded phase or between cytokine levels at baseline and at the end of the add-on phase (table 3).

Table 3. Plasma cytokine levels

	Baseline	Blind phase: active control group	Blind phase: treatment group	End of add-on	p^a	p^b
IL-1 β , pg/ml	0.06 (0.06–8.00)	0.14 (0.06–1.30)	0.06 (0.06–0.62)	0.14 (0.06–1.92)	0.87	0.73
IL-6, pg/ml	1.16 (0.23–9.57)	1.09 (0.25–17.39)	1.27 (0.30–10.13)	1.08 (0.37–18.57)	0.64	0.79
IL-10, pg/ml	0.78 (0.39–6.87)	0.78 (0.39–5.46)	0.78 (0.39–7.53)	0.78 (0.39–14.58)	0.50	0.10

Values are medians (range).

^a Comparison of active control and treatment groups by the Mann-Whitney U test.

^b Comparison of levels at baseline and at the end of the add-on phase by a related-samples Wilcoxon signed-rank test.

Seizure Frequency Reduction and Cytokine Changes

Four patients were excluded from seizure frequency analysis due to incomplete seizure diaries. Twenty-two percent ($n = 8$) of the patients experienced $\geq 50\%$ seizure frequency reduction. The change in seizure frequency during the add-on phase did not correlate with changes in cytokine levels.

Cognitive Function and Cytokine Changes

Fourteen patients could not participate in cognitive testing due to cognitive and/or physical limitations. The

IQ tended to increase from a mean score of 55.1 (baseline) to 58.2 (end of the add-on phase) ($p = 0.08$, paired t test). The effects on cognition were positively correlated with a change in IL-1 β ($\tau = 0.42$, $p < 0.01$): the larger the IL-1 β increase, the greater the improvement of IQ (fig. 2). Changes in IL-6 and IL-10 did not significantly correlate with changes in IQ.

Predictive Value of Baseline Cytokines

Baseline plasma levels of IL-6 predicted seizure frequency reduction during the add-on phase [$R^2 = 0.105$ (1,

35), $p = 0.050$]: lower IL-6 levels were associated with more seizure frequency reduction. Pre-implantation CSF levels did not predict seizure frequency reduction.

Discussion

To our knowledge, this exploratory study is the first study to evaluate the effects of VNS on cytokine levels of children. VNS reduced seizure frequency by more than 50% in 22% of the patients. We were unable to confirm our hypothesis that VNS evokes an anti-inflammatory reaction since we did not find a statistically significant effect of VNS on interictal plasma cytokine levels. Similar to our results, two studies in adults suffering from medically refractory epilepsy did not show significant changes in blood levels of IL-6, IL-10, and tumor necrosis factor- α (TNF- α) [19, 20]. A study in adult depressed patients, in contrast, showed that 3 months of VNS increased plasma levels of IL-6, TNF- α , and transforming growth factor- β [17].

Several reasons might explain the absence of the hypothesized VNS-induced anti-inflammatory reaction. Firstly, VNS did not alter interictal cytokine levels. Since epilepsy patients are known to have altered interictal cytokine levels [32–34], we specifically determined whether VNS changed cytokine levels more than 12 h after a seizure. Nevertheless while these cytokine levels were unchanged, we cannot exclude the possibility that VNS treatment inhibits cytokine changes at a different time point. For example, the chosen interval might not have been sufficient for cytokines to return to their baseline levels. On the other hand, if VNS only inhibits cytokine expression in the immediate postictal phase, this effect would not be detected using the present study design. Although it is technically challenging, future studies should compare cytokine levels immediately before and after a seizure [35, 36].

Furthermore, although VNS did not alter plasma cytokine levels, it may still have affected CNS cytokine levels. CSF cytokine levels are probably the best representation of CNS cytokine levels available but, for ethical reasons that limit CSF collection in children, CSF was only obtained at one time point which made longitudinal monitoring impossible.

There are several limitations, which might account for the lack of changes in cytokine levels. For example, the study included a relatively small number of patients who suffered from different epilepsy syndromes. This might have limited the possibility of detecting significant effects

as there is evidence that plasma cytokine concentrations differ between various etiologies and pathologies [33, 35, 37]. In addition, the relatively low responder rate may have affected an association between the anticonvulsive effectiveness of VNS and any cytokine changes.

Contrary to seizure frequency reduction, changes in IQ were correlated with altered levels of IL-1 β : a larger increase in IL-1 β was associated with a larger improvement of IQ. IL-1 β is a pro-inflammatory cytokine which has pro-convulsive properties [38]. It is also implicated in cognitive function and has been shown to have both beneficial and detrimental effects on learning and memory [39]. However, as no causality can be inferred from the current analysis, future studies should explore whether changes in IL-1 β actually contribute to improved cognition upon VNS.

Our study revealed that low baseline IL-6 levels correspond to a greater reduction in seizure frequency and thus predict the clinical response. IL-6 is a cytokine with both pro- and anti-inflammatory characteristics that mediates the acute phase reaction and fever. The predictive value of IL-6 is particularly interesting since IL-6 is the most consistently and chronically changed circulating cytokine in epilepsy [32–34]. However, other investigators failed to detect any cytokine changes that were of potential predictive value [18], possibly due to the fact that the changes were evaluated in epileptic adults using whole blood samples in vitro that were stimulated with lipopolysaccharide. In addition, studies in adults cannot be compared to studies in children without taking into account the age dependency of cytokine availability [40].

Conclusion

VNS decreases seizure frequency by more than 50% in 22% of pediatric epilepsy patients and may improve IQ. Larger increases in IL-1 β are related to a greater improvement of IQ. Low baseline IL-6 levels predict a greater reduction in seizure frequency. Thus, the determination of IL-6 prior to VNS treatment may be a valuable tool to aid responder identification in the future.

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