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The effects of vagus nerve stimulation on tryptophan metabolites in children with intractable epilepsy

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ABSTRACT

Background: The mechanism of action of vagus nerve stimulation (VNS) in intractable epilepsy is not entirely clarified. It is believed that VNS causes alterations in cytokines, which can lead to rebalancing the release of neurotoxic and neuroprotective tryptophan metabolites. We aimed to evaluate VNS effects on tryptophan metabolites and on epileptic seizures and investigated whether the antiepileptic effectiveness correlated with changes in tryptophan metabolism.

Methods: Forty-one children with intractable epilepsy were included in a randomized, active-controlled, double-blind study. After a baseline period of 12 weeks, all children underwent implantation of a vagus nerve stimulator and entered a blinded active-controlled phase of 20 weeks. Half of the children received high-output (therapeutic) stimulation ($n = 21$), while the other half received low-output (active control) stimulation ($n = 20$). Subsequently, all children received high-output stimulation for another 19 weeks (add-on phase). Tryptophan metabolites were assessed in plasma and cerebrospinal fluid (CSF) by use of liquid chromatography–tandem mass spectrometry (LC–MS/MS) and compared between high- and low-output groups and between the end of both study phases and baseline. Seizure frequency was recorded using seizure diaries. Mood was assessed using Profile of Mood States (POMS) questionnaires.

Results: Regarding tryptophan metabolites, anthranilic acid (AA) levels were significantly higher at the end of the add-on phase compared with baseline ($p = 0.002$) and correlated significantly with improvement of mood ($\tau = -0.39$, $p = 0.037$) and seizure frequency reduction ($\tau = -0.33$, $p < 0.01$). No significant changes were found between high- and low-output groups regarding seizure frequency.

Conclusion: Vagus nerve stimulation induces a consistent increase in AA, a neuroprotective and anticonvulsant tryptophan metabolite. Moreover, increased AA levels are associated with improvement in mood and reduction of seizure frequency.

1. Introduction

Vagus nerve stimulation (VNS) is an adjunctive treatment for therapy-resistant epilepsy. The mode of action of VNS is still largely unknown. The possible underlying mechanism of action of VNS may be related to its influence on neuronal networks affecting deep brain structures such as the locus coeruleus, hypothalamus, and thalamus [1,2]. More specifically, VNS might have immune-modulating effects as the vagus nerve has antiinflammatory properties, affecting the hypothalamic–pituitary–adrenal axis and the release of corticosteroids [3]. The therapeutic field of VNS in inflammatory diseases is still expanding, and both pro- and antiinflammatory effects of VNS have been reported [4–6].

Changes in cytokine levels from VNS may affect metabolism of the serotonin (5-HT) precursor tryptophan. This metabolism is influenced by proinflammatory cytokines: the enzyme indoleamine 2,3-dioxygenase (IDO) is activated under the influence of proinflammatory cytokines, which results in tryptophan entering the 'tryptophan metabolic pathway' instead of the formation of 5-HT (for a simplified overview, see Fig. 1) [6]. Kynurenine (KYN) formation increases because of this shift in tryptophan metabolism.

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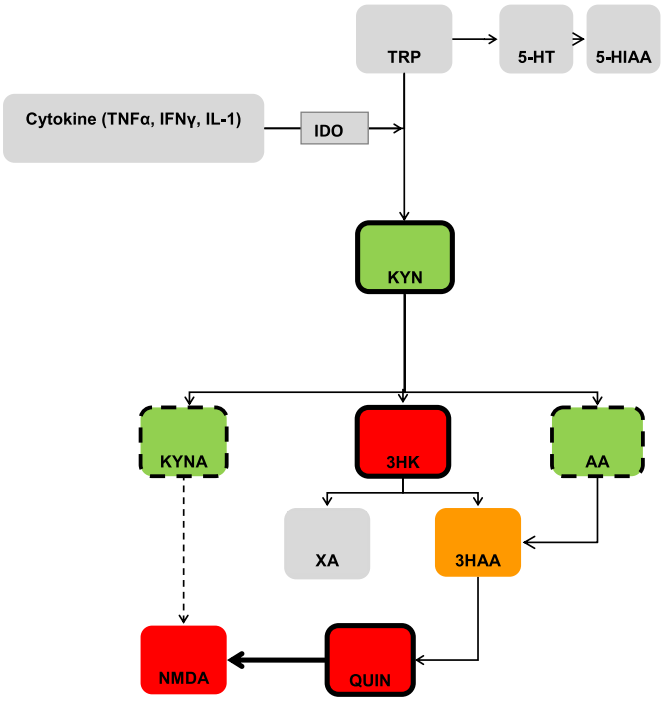

Fig. 1. Metabolites of tryptophan pathway. This figure depicts the tryptophan breakdown metabolic pathway. IDO activity in extrahepatic tissues, such as blood and brain, is enhanced by proinflammatory cytokines. This condition increases KYN formation. Green: neuroprotective metabolites. Red: neurotoxic metabolites. Orange: probably neurotoxic. Dashed thick outline: anticonvulsant effects. Thick outline: proconvulsant effects. Dashed arrow: antagonist; thick arrow: agonist. Abbreviations: TRP = tryptophan; 5-HT = serotonin; 5-HIAA = 5-hydroxyindoleacetic acid; IDO = indoleamine 2,3-dioxygenase; KYN = kynurenine; KYNA = kynurenic acid; 3HK = 3-hydroxykynurenine; AA = anthranilic acid; XA = xanthurenic acid; 3HAA = 3-hydroxyanthranilic acid; NMDA = N-methyl-D-aspartate; QUIN = quinolinic acid.

Proinflammatory cytokines appear to be present in the epileptic brain [7,8], and the tryptophan metabolic pathway may be active in epilepsy [9]. Indeed, some of the tryptophan metabolites influence seizure development, and, therefore, anticonvulsant effects of VNS may be partially explained by its effect on this pathway [10,11]. Kynurenine and 3-hydroxykynurenine (3HK) are proconvulsant, while kynurenic acid (KYNA) and anthranilic acid (AA) are anticonvulsant [12–16]. Kynurenine, kynurenic acid, and anthranilic acid are presumed to be neuroprotective, while 3HK is presumed to be neurotoxic. Whether the remaining metabolites 3-hydroxyanthranilic acid (3HAA) and xanthurenic acid (XA) are proconvulsant, anticonvulsant, neurotoxic, or neuroprotective remains to be established [14–18].

The only randomized controlled trial conducted in children concluded that the effect of VNS on seizure frequency in children with intractable epilepsy is limited [19]. Nonrandomized studies on children treated with VNS report a variable reduction in seizure frequency, ranging from 0 to 90% [20–28].

A study evaluating the VNS-induced changes in tryptophan metabolism in children has not been published so far.

We hypothesize that VNS in children with refractory epilepsy affects tryptophan metabolism, resulting in reduced neurotoxic and proconvulsant metabolites and in increased neuroprotective and anticonvulsant metabolites.

In the present study, we aimed at (1) evaluating the effect of VNS on the tryptophan metabolic pathway in children, (2) evaluating if the therapeutic effect of VNS is associated with changes on the tryptophan pathway in children, and (3) evaluating whether a baseline profile of tryptophan metabolites can predict the clinical response to VNS in children.

2. Methods

2.1. Study design

This randomized, active-controlled, double-blind, add-on study was divided into a baseline period (12 weeks), a blinded treatment phase (20 weeks), and an add-on phase (19 weeks). Following a baseline period of 12 weeks, all children were implanted with a vagus nerve stimulator. During the blinded treatment phase, children received either high (therapeutic) or low (active control) stimulation. This active control group was incorporated to protect the blinding, because participants can notice the stimulation, and to control for the surgery and the presence of an electrode. After the blinded phase, all children received 19 weeks of therapeutic stimulation during the add-on phase. Blood samples were collected before implantation of the stimulator, at the end of the blinded phase after 20 weeks of stimulation, and, finally, at the end of the add-on phase after 39 weeks of stimulation. Cerebrospinal fluid (CSF) samples were collected only once when the children were under general anesthesia right before VNS implantation surgery. Seizure frequency was recorded throughout the study making use of a seizure diary. Mood was assessed using Profile of Mood States (POMS) questionnaires at baseline, at the end of the blinded phase, and at the end of the add-on phase [29] (see Fig. 2 for the study design).

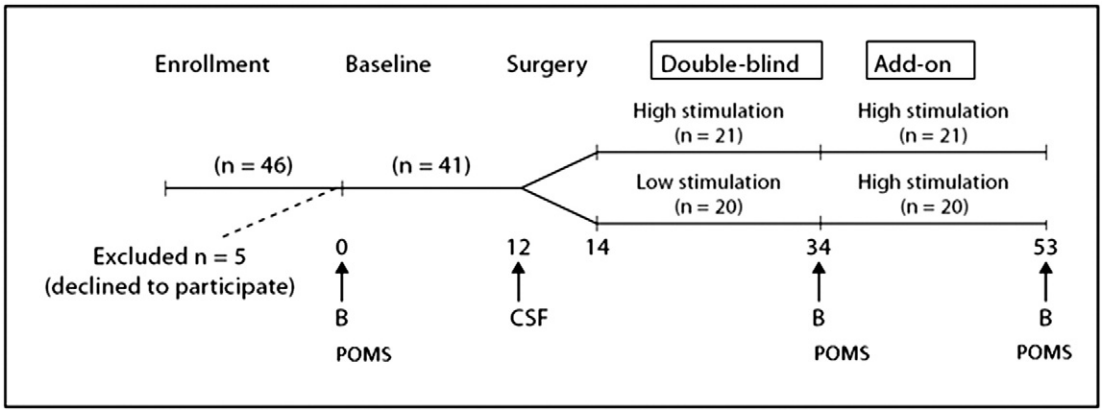

Fig. 2. Study design. Arrows indicate data and sample collection: B = blood; CSF = cerebrospinal fluid; POMS = Profile of Mood States questionnaire. Numbers above arrows indicate weeks of study duration.