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ORIGINAL ARTICLE

Effect of transcutaneous vagus nerve stimulation on muscle activity in the gastrointestinal tract (transVaGa): a prospective clinical trial

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Abstract

Purpose Postoperative ileus (POI) is a common complication after abdominal surgery. Invasive stimulation of the cervical vagus nerve is known to reduce inflammatory response and ameliorated POI after surgery in a mouse model. However, the transcutaneous vagus nerve stimulation (tVNS) is a possible non-invasive approach. In this clinical study, we aimed to investigate the effect of tVNS on the activation of the stomach muscle in humans.

Methods Patients requiring open laparotomy were screened for this prospective proof of concept clinical study. After open laparotomy, muscle activity of the stomach was measured by a free running electromyography (EMG) before and during tVNS on the ear. Frequency and amplitude of compound gastric action potentials were the electrophysiological parameters we assessed to reveal the changes in electro motor gastric activity. Gastrin levels as a surrogate marker for vagus nerve activation was analyzed before, 1 and 3 h after tVNS.

Results Fourteen patients were included, no severe adverse events and no medical device related adverse events occurred. tVNS led to significant reduction of action potential frequency and significant elevation of action potential amplitude in the stomach compared to control. Gastrin levels were significantly elevated 3 h after tVNS compared to levels before tVNS.

Conclusion Application of tVNS is a safe and feasible procedure during surgical intervention. Our results provide evidence that tVNS activates efferent visceral vagal fibers. Therefore, this low risk and easy to perform method could be useful to prevent postoperative ileus.

Clinical trial register number DRKS00013340.

Keywords Transcutaneous vagus nerve stimulation · Neurogastroenterology · Postoperative ileus · Clinical trial

Introduction

Abdominal surgery leads to a transient phase of impaired bowel motility, which could lead to a postoperative ileus (POI) accompanied by vomiting, nausea, intolerance to food, and delayed defecation [1, 2]. This causes a significant increase of morbidity and additional health care costs [3]. Thus far, treatment with erythromycin, cholecystokinin, neostigmine, or lidocaine showed no clear evidence for an amelioration of POI [4]. POI is induced by intestinal manipulation (IM) during surgery, followed by an immunoreaction causing a transient paralysis of the muscular layer of the intestine [5].

The vagus nerve increases motility in the stomach and intestine, initiating excitatory stimuli within the smooth muscle cells under physiological conditions [6]. Furthermore, the

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vagus nerve was shown to reduce inflammatory response in a sepsis model, introducing the concept of the cholinergic anti-inflammatory pathway [7, 8]. An invasive vagus nerve stimulation (VNS) significantly reduced the inflammatory reaction in the intestine and ameliorated POI after surgery in a mouse model [9]. Cailotto et al. showed that intestinal inflammation is reduced by a local, indirect acetylcholine release from enteric neurons, which inhibits macrophage activation via α7 nicotinic acetylcholine (ACh) receptors [10] and dampens intestinal inflammation in POI [11]. In contrast, vagotomy showed an aggravation of inflammation [12–14]; however, invasive vagus nerve stimulation involves an additional intervention with surgical exposure of the vagus nerve. As POI is a transient condition, the risk of this intervention is definitely not justified.

In contrast, the transcutaneous vagus nerve stimulation of the auricular branch is a non-invasive approach. In our preliminary study with a murine POI model, we could show that transcutaneous vagus nerve stimulation (tVNS) reduces intestinal inflammation and improves POI [15]. In this study, we aimed to investigate if transcutaneous stimulation can be conducted to visceral fibers in humans. Therefore, we evaluated the effect of tVNS on the activation of the stomach muscle in humans.

Material and methods

A single-center prospective study was performed at the department of surgery, university hospital of Bonn between February 2015 and May 2016, following approval of the federal institute for Drugs and Medical Devices (CIV-1504-013367) and the local ethical board (179/15-MPG). It was registered at the German clinical trials register: DRKS00013340. Patients above the age of 18 and under the age of 70, who required an elective, open laparotomy were enrolled in the study. Exclusion criteria included: the absence of a written, informed consent, gastritis, gastropareses, diabetes, severe comorbid disease, pregnancy, neuropathological disease, emergency surgery, and intake of anticholinergic medication. Sixteen patients were screened and 14 patients were finally enrolled in the study.

The trial protocol

Patients, requiring open laparotomy, were screened and included 21 days to at least 24 h prior surgery. Included patient were anesthetized via total intravenous anesthesia (TIVA) without relaxation. After laparotomy, needle electrodes (SDN Elektrode Trigon®, Inomed, Emmendingen, Germany) were placed pairwise parallel with a distance of approximately 1–2 cm into exposed areas of the stomach (depending on the surgery) without penetrating the stomach wall. Wires were connected to the ISIS intraoperative neuromonitoring recorder (ISIS IOM System®, Inomed). Neutral electrode was placed subdermal at the scapula. EMG of accessible areas of the stomach (fundus, corpus, and pylorus) was recorded for 5 min. before stimulation as a baseline control. A transcutaneous, bipolar stimulation probe (Stimulationssonde 522,015, Inomed) was attached to the cymba conchae of the right ear. tVNS was performed for 10 min on the ear (10 mA, 25 Hz, and 250 μs). During tVNS, EMG was recorded again. EMG was performed under the supervision of a neurosurgeon with experience in intraoperative neuromonitoring and technical support by Inomed. Additionally, heart frequency and blood pressure were monitored constantly to control any hemodynamic instability. Electrodes were removed after the stimulation and surgery was continued as planned before. Before stimulation, 1 and 3 h after tVNS, blood samples were gathered to measure gastrin levels. Patients were re-examined daily after surgery for 3 days (Fig. 1).

EMG processing

The EMG of each site for every patient was evaluated individually by hand with the spike 2 Version 8 software after it was passed through a low band filter. The period with the basal EMG before stimulation and the period during stimulation were assessed separately. Action potentials were counted and frequency was calculated as the number of action potential during the time between the beginning of the first action potential till the ending of the last recorded action potential. Mean amplitude was computed automatically for each patient for the basal and stimulation period after individual adaptation of the threshold for action potential recognition.

Gastrin measurement

As a surrogate marker of vagus nerve activity, gastrin levels were measured before and after tVNS. Blood samples were taken during control EMG before tVNS, 1 and 3 h of tVNS. Blood samples were collected in tubes, containing clot activators (S-Monovette® 4.7 ml Z-Gel, Sarstedt AG & Co., Nuernbrecht, Germany) and were analyzed by a chemiluminescence immunoassay (Siemens Immulite, Siemens, Muenchen, Germany).

Statistics

Statistical analysis was performed using a one-way ANOVA with Bonferroni post-hoc-test or paired Student's t test with Prism 5.02 software (GraphPad, La Jolla, USA). Data were considered statistically significant at p values < 0.05 (*), < 0.01 (**) and < 0.001 (***).

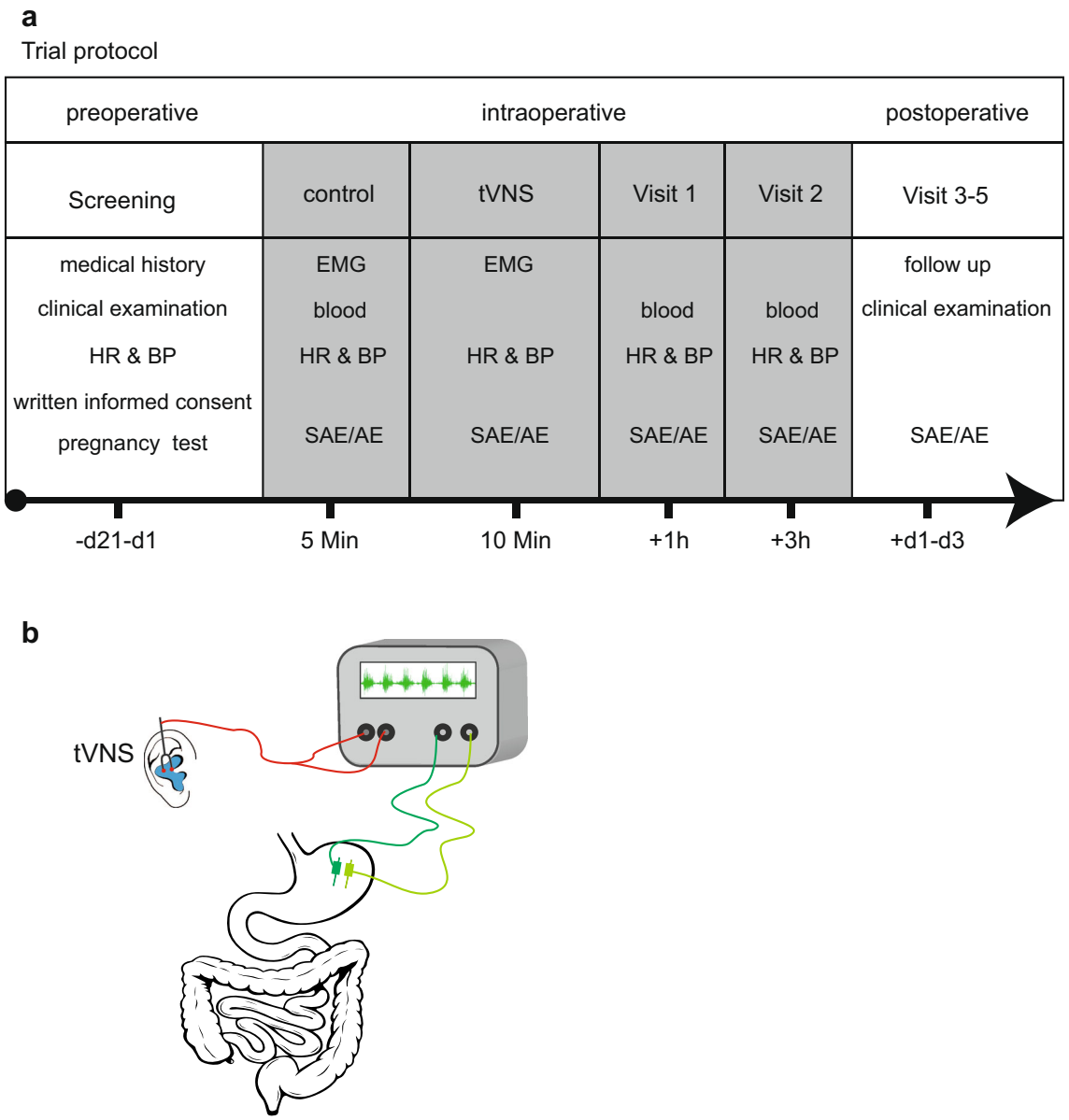

Fig. 1 **A** Trial protocol. **B** Schematic representation of intraoperative EMG. The cymba conchae of the ear (blue) was stimulated transcutaneous via a bipolar stimulation probe (red). Needle electrodes were placed pairwise parallel with a distance of approximately 1–2 cm into exposed areas of the stomach (depending on the surgery) without penetrating the stomach wall (green). Wires were connected to the ISIS intraoperative neuro-monitoring recorder (gray). EMG of the stomach was recorded before and during transcutaneous stimulation of the ear

Results

Sixteen patients requiring open abdominal surgery were screened and 14 patients were included in the trial. Two patients were excluded, one patient's surgery had to be postponed and one patient had insufficient knowledge of the German language to fully participate in the trial. All 14 patients underwent EMG measurement before and during tVNS (Table 1).

Safety and feasibility

No patient experienced hemodynamic instability during tVNS. Twelve AEs occurred during the trial to nine patients,

Table 1 Demographic data

Age (years)	57.6 ± 10.5
Sex	m = 6, w = 8
Operative procedure	- 4× pylorus-preserving pancreaticoduodenectomy - 2× open partial liver resection - Open splenectomy - Open gastrectomy - Open left colectomy - Open anterior rectum resection - Open extended right hemihepatectomy - Open ileocecal resection - Open ventral hernia repair

the complications were caused by the surgical procedures. There was no AE related to the device. No SAE occurred during the trial (Table 2).

Clinical outcomes

Electromyography

To investigate if tVNS activates efferent vagal fibers, muscle activity of the stomach was measured intraoperatively by an electromyography (EMG) of the stomach muscle before (control) and during tVNS (intervention) in different areas of the stomach (Fig. 2). In the corpus (Fig. 2a) and pylorus (Fig. 1b), the number of action potentials per minute (AP) was significantly decreased during tVNS compared to control (corpus: 2.98 ± 0.63 vs 3.69 ± 0.93 AP/min, $p < 0.05$; pylorus: 3.19 ± 1.15 vs 3.61 ± 1.18 AP/min, $p < 0.001$). The amplitude was increased during tVNS compared to control within the corpus; however, not significantly (0.113 ± 0.05 vs 0.09 ± 0.04 mV, $p = 0.067$) (Fig. 2c). More importantly, the amplitude in the pylorus increased significantly during tVNS compared to control (0.25 ± 0.19 vs 0.19 ± 0.14 mV, $p < 0.05$) (Fig. 2d).

Heart rate and mean blood pressure

As the stimulation of the vagus nerve can lead to bradycardia and hypotension, the heart rate and mean blood pressure was measured before and during the EMG. There were no significant changes in heart rate (72.93 ± 17.55 vs 67.07 ± 15.96 bpm) or mean blood pressure (50.29 ± 12.52 vs 50.07 ± 13.87) during tVNS compared to control (Fig. 3a/b).

Table 2 List of AEs

Patient	Visit	AE	Surgery	Device related	Outcome
03	4	Pain due to insufficient peridural analgesia		No	Resolved
03	5	Pain due to insufficient epidural analgesia		No	Resolved
04	3	Postoperative delirium		No	Resolved
06	4	Fever		No	Resolved
08	4	Postoperative bile duct fistula		No	Resolved
09	5	Pancreatic fistula		No	Ongoing
11	3	Back pains		No	Resolved
11	5	Peranal hemorrhage		No	Resolved
12	5	Pancreatic fistula		No	Ongoing
12	5	Postoperative bleeding		No	Resolved
13	5	Vomiting		No	Resolved
14	3	Vomiting		No	Resolved

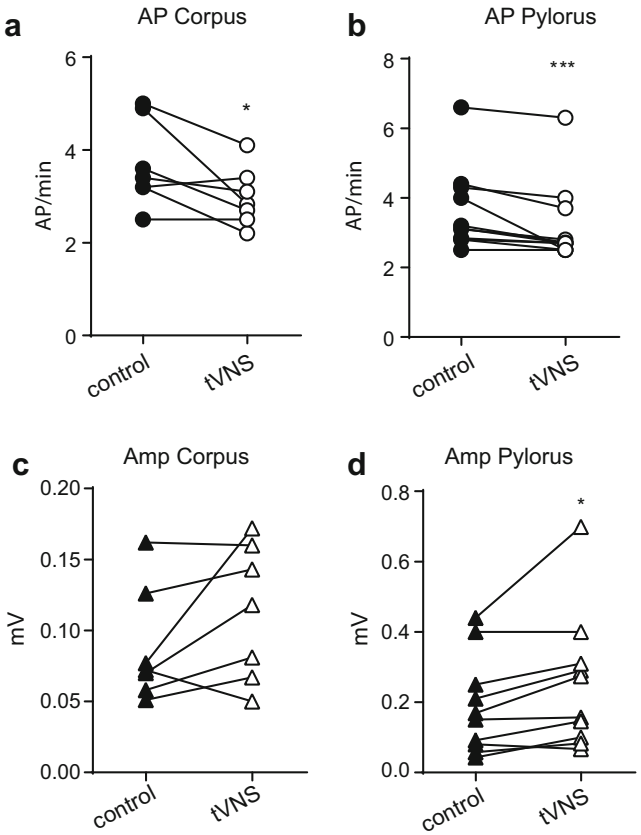

Fig. 2 EMG recording before and during tVNS. After open laparotomy, intraoperative EMG was performed before and during tVNS. The amount of (**a**/**b**) action potentials (AP) and (**c**/**d**) amplitudes (Amp) were measured within (**a**/**c**) the corpus and (**b**/**d**) the Pylorus. Statistical analysis was performed by a paired Student's *t* test. Each line represents one patient (corpus: $n = 7$, pylorus: $n = 10$), * = $p < 0.05$, ** $p < 0.01$ and *** = $p < 0.001$

Gastrin measurements

As gastrin secretion depends on the vagus nerve stimulation, gastrin levels within the blood serum were measured before and after tVNS. The gastrin levels were not significantly elevated 1 h after stimulation compared to control (86.37 ± 73.05 vs 82.83 ± 79.05 pg/ml, $p = 0.28$); however, 3 h after tVNS, gastrin levels were significantly increased compared to control (132.2 ± 133.8 vs 82.83 ± 79.05 pg/ml, $p < 0.05$) (Fig. 3c).

Discussion

The anti-inflammatory effect of the vagus nerve is an attractive strategy against inflammatory diseases. In most approaches, the vagus nerve was stimulated invasively cervical [16] or sub-diaphragmal [17]. In our approach, vagus nerve stimulation was performed non-invasively via the auricular branch. Previously, we showed that tVNS reduced inflammatory reaction of the intestine and ameliorated POI significantly in mice [15]. To detect if transcutaneous stimulation can be

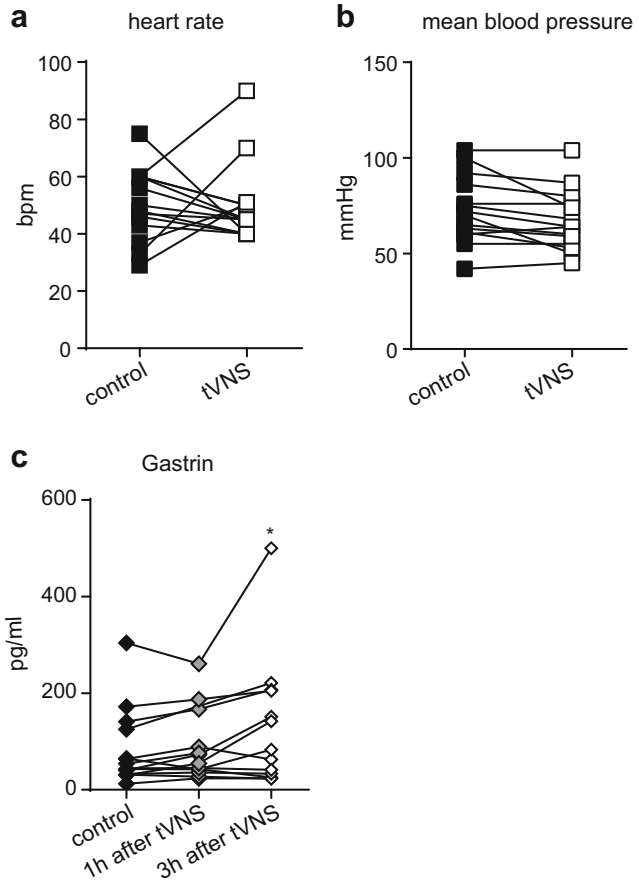

Fig. 3 After open laparotomy, intraoperative EMG was performed before and during tVNS. **a** The heart rate and **b** blood pressure were measured during tVNS and compared to control (before tVNS). **c** Gastrin concentration within the blood was measured before tVNS (control) and after tVNS at different time points. Statistical analysis was performed by (**a**/**b**) a paired Student's *t* test or **c** a one-way ANOVA followed by Bonferroni post-hoc test. Each point represents one patient ((**a**/**b**) n = 14, (**c**) $n = 13$) * = $p < 0.05$, ** $p < 0.01$ and *** = $p < 0.001$

conducted to visceral fibers in humans, muscle activity of the stomach was measured via running EMG.

To the best of our knowledge, this is the first report examining the effect of tVNS on the muscle activity of the stomach in humans. Of note, we did not investigate the effect of tVNS on the intestinal motility or inflammatory reaction. We could show that tVNS is feasible and well-tolerated. No device related major or minor side-effects were observed. Minor side effects as irritation of the skin and itching were described under tVNS application for epilepsy, but were reversible upon completion (vigilance data, cerbomed GmbH). As we performed tVNS during general anesthetic in this trial, patients did not experience any local inconvenience. Our data show that electric stimulation of efferent fibers of the vagus nerve resulted in an alteration of gastric EMG. Surprisingly, the frequency of APs decreased during tVNS compared to the prior control period. However, there was a significant increase of the amplitude of the AP supporting the hypothesis of an improved propulsion during tVNS. In the stomach, the vagal effect on the electrophysiological activity of intestinal cajal cells and smooth muscle cells has not been explored yet, but vagal efferent fibers could lead to a synchronization of the neuro-myenteric network [18] resulting in an effective propulsion explaining the reduction of frequency of APs and elevation of the amplitude. Interestingly, the stimulation of the vagus nerve is reported to lead to a desynchronization in the brain preventing epileptic seizures [19]. Moreover, the serum gastrin concentration, as a surrogate marker for functional vagus nerve activation [20], significantly rose after tVNS compared to control, providing further evidence for vagal activity after tVNS. Even though no manipulation of the gut was performed prior and during stimulation, we cannot exclude that laparotomy alone induces the rise of gastrin levels; however, Xiaoli et al. [21] observed no significant changes in gastrin serum levels during surgery, suggesting that surgery alone does not activate gastrin release. Invasive vagus nerve stimulation gets more relevant for treatment in humans as clinical trials of inflammatory diseases like morbus crohn [22, 23] and rheumatoid arthritis [24] showed a significant reduction of inflammation and improvement of disease progression; however, implantation of a stimulation device and probe would not be ethically responsible for transient pathological conditions such as POI. For that very reason, tVNS could be a non-invasive method to modulate immune response after abdominal surgery. Another approach was investigated by Stakenborg et al. using an intraoperative, abdominal vagus nerve stimulation in a clinical setting, yielding promising data to reduce inflammatory response after surgery [17]. Also in this approach, there are limitations where intraoperative stimulation is not feasible (peritoneal adhesion formation, obesity) and a sub-diaphragmal exposure of the vagus nerve requires additional surgical trauma. In contrast, tVNS is a more feasible and safe application. Our data suggest that tVNS could be a potential tool in prevention of POI and warrant therefore further investigations with a prospective, randomized clinical trial.

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Compliance with ethical standards

Conflicts of interest GH received research grant support by PlantTec Medical GmbHSW and JCK received royalties from Wolters Kluwer for writing articles about POI in UpToDate® resource tool.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the

institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standard. The study was approved by the federal institute for Drugs and Medical Devices (BfArM) (CIV-1504-013367) and the local ethical board (179/15-MPG).

Informed consent Informed consent was obtained from all individual participants included in the study.

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