Vagotomy and Gastric Tumorigenesis

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Abstract

Vagotomy reduces gastric acid secretion and was therefore introduced as a surgical treatment for peptic ulcers in the 1970s. Later, it was replaced by acid reducing medication, such as histamine type 2 (H2) receptor antagonists and proton pump inhibitors (PPIs). A large body of evidence has indicated that drug-induced hypochlorhydria per sedoes not increase the risk of gastric cancer. Early studies on the effects of vagotomy in chemically-induced rodent models of gastric cancer reported an increased risk of developing gastric cancer. This was most likely due to a delayed gastric emptying, which later has been accounted for by including an additional drainage procedure, e.g. pyloroplasty. In a recent study using three different mouse models of gastric cancer (including genetically engineered, chemically-induced and Helicobacter pylori-infected mice), either unilateral vagotomy or bilateral truncal vagotomy with pyloroplasty was found to significantly attenuate tumorigenesis in the denervated side of the stomach at early preneoplastic stages as well as at later stages of tumorigenesis. Consistently, pharmacological denervation using botulinum toxin A or muscarinic acetylcholine receptor 3 (M3R) blockade inhibited tumorigenesis. Moreover, it was found that recurrence of gastric cancer was reduced in patients following vagotomy. Thus, these new findings suggest the potential treatment strategies to target the nerve, neurotransmitters, corresponding receptors and their downstreamsignaling pathways for the malignancy.

Keywords

Peptic ulcer; Vagotomy; Vagus nerve; Botulinum toxin A; Denervation; Gastric tumorigenesis; Stem cell signaling; Muscarinic receptors

1. Introduction

1.1 History of Vagotomy

The anatomy of the vagus nerve was first described by Galen in the second century AD, and further investigated by surgeons during the first quarter of the century [1]. The therapeutic possibilities of vagotomy of the stomach were of great interest to clinicians. Vagotomy, known as the surgical resection or cutting of parts of the vagus nerve that innervates the stomach, actually comprises at least three different types of surgery that include truncal, selective and highly selective vagotomies [2, 3]. The vagus nerve divides into two branches, and each branch innervates one side of the stomach. Vagotomy is often performed together with other surgical procedures, such as pyloroplasty, gastric resection or antrectomy [2]. In the surgical management of peptic ulcers, vagotomy has been an essential component as it reduces gastric acid secretion [3]. The history of peptic ulcer treatment began in the 19th century, when William Prout discovered hydrochloric acid within the gastric lumen, enabling him to relate the presence of hydrochloric acid in the stomach to the occurrence of dyspepsia [1]. The role of the vagus nerve in the control of gastric acid secretion was identified in 1814 by Benjamin Brodie and later elaborated on by Ivan Pavlov [4]. In the early 1900s, the standard operation for the treatment of gastric and duodenal ulcers was either a pyloroplasty without vagotomy, or a gastroenterostomy. Pyloroplasty was later surpassed by gastroenterostomy as the treatment of choice, which then was superseded by gastric resection, comprising 80 % of procedures by the early 1950s [4]. Based on a better understanding of the mechanism behind gastric acid secretion, subtotal gastrectomy was used for the treatment of ulcers for the next several decades. The effects of vagotomy on acid secretion and gastric motility were established in the early 20th century .In 1921, Andre Latarjet applied knowledge of the anatomy of the vagus nerve clinically by performing an anatomically complete vagotomy for dyspepsia [4]. By the year 1938, Komarov had confirmed the existence of gastrin and its stimulatory effects on acid secretion, and in 1943,

Lester Dragstedt reported that subdiaphragmatic vagotomy favorably influence the clinical course of duodenal ulcer [1, 3]. The surgical approach at this time designed largely to reduce acid secretion [4]. By incorporating both vagotomy and antrectomy (which removed acid stimulating gastrin cells), acid production would be effectively reduced, thus continuing the "no acid, no ulcer" dogma of the time [4]. Dragstedt also introduced into clinical practice the combination of truncal vagotomies with a pyloroplasty. After the World War II, this paradigm of surgical control of acid secretion served as the basis of treatment of peptic ulcer disease [5]. With the introduction of selective vagotomies, e.g. proximal gastric vagotomy without pyloroplasty, both decreased acid secretion and less-effected motility were achieved. The treatment of choice for duodenal ulcers included diverse vagotomies with or without antrectomy. Type I gastric ulcers were preferentially treated either by partial gastrectomy or by performing antrectomy with gastroduodenostomy (Bilroth I). Currently, gastrectomy to treat peptic ulcers is only used if there is a defined risk for gastric cancer in an unhealed gastric ulcer. Gastrectomy may also be performed in the case of recurrent or therapy-resistant pre-pyloric ulcers [6]. In the late 1970s, the use of H_2 blockers was introduced and later proton pump inhibitors emerged [7]. A remarkable parallel can be seen between the developments of the two approaches in the 1970s; highly selective vagotomy was introduced as a new alternative to partial gastrectomy in surgical treatment of peptic ulcers in 1971. Later during the same decade, surgery was performed less often as a result of improved H_2 receptor antagonist treatment [8, 5]. After 1984, the etiological role of *Helicobacter pylori* (*H. pylori*) in the peptic ulcer disease was recognized and with this came a better understanding of the pathophysiology of peptic ulcers, and thus treatment changed accordingly [1, 4]. Research has shown a clear role for *H. pylori* in the development of not only peptic ulcers, but also gastric cancer, a topic extensively reviewed by David Y. Graham in 2014 [9]. Graham also recently published an updated overview of effective therapies and possible benefits associated with *H. pylori* eradication to prevent gastric carcinogenesis [10].

1.2 Risk of Gastric Tumorigenesis after Inhibition of Gastric Acid Secretion in Patients with Peptic Ulcers

Vagotomy had been used as a surgical treatment for peptic ulcers because it reduces gastric acid secretion. The secondary hypochlorhydria and hypergastrinemia have been thought to be risk factors for gastric tumorigenesis. In fact, vagotomy is much less powerful than H_2 receptor inhibitors or proton pump inhibitors in terms of the inhibition of acid secretion and the secondary elevation of circulating gastrin levels. Conceivably, vagotomy would unlikely increase the risk for gastric cancer if H_2 receptor inhibitors or PPIs were without the effect. Indeed, there is no evidence for a carcinogenic effect after long-term use of H_2 antagonists. It was reported that patients had an excess gastric cancer incidence during the first 4 years of treatment [11], but this was almost certainly due to initially misdiagnosed cancer [11-13]. Also, in a study investigating the risk of gastric cancer among cimetidine users it was reported that there was an increased long-term risk of cancer in female but not male patients [7]. The sample size was too low to reach any conclusion as there were only 6 women diagnosed with gastric cancer in that study. PPIs have become one of the most commonly used medications worldwide, as they are currently the best treatment of choice for several gastric acid-related gastrointestinal disorders because they are effective in reducing the acid secretion and have no long-term adverse effects [14, 15]. It is not surprising that there has been debated whether PPI therapy increases, decreases or has no effect on gastric cancer risk [16-19].

It has been well recognized that there is a positive association between gastric ulcer disease and the risk of developing gastric cancer, and a negative association between duodenal ulcerations and the risk of gastric cancer [20]. Thus, it would be of interest to find out whether vagotomy that was initially performed in gastric ulcer patients could increase the risk of gastric cancer.

1.3 Risk of Gastric Cancer after Vagotomy in Humans and Animal Models

Surgical treatment such as partial gastrectomy or vagotomy has traditionally been thought to be associated with an increased risk of gastric cancer due to the secondary hypochlorhydric conditions in the stomach [21-26]. A prospective study, which included 1495 patients, used a pathological gastritis index to evaluate the risk of developing gastric cancer after peptic ulcer surgery. Patients who underwent either truncal vagotomy with drainage or partial gastrectomy displayed atrophic gastritis index 2.3 ± 0.08 and 2.6 ± 0.1, respectively, in comparison with nonoperated patients with dysplasia at 1.8 ± 0.08. Thus, there was no evidence to that vagotomy or partial gastrectomy could lead to either an increased risk of cancer or precancerous lesions [27]. Furthermore, a 20+-year follow-up study, which included 5018 patients who underwent gastric surgery or truncal vagotomy with either drainage or gastroenterostomy, showed that of the 5 gastric ulcer patients who underwent vagotomy, 1 case of cancer was observed compared to 0.6 cases expected during the first 19 postoperative years, thus yielding "a mortality rate of 1.7". Apparently, there was no significant increase in mortality during the 20-year follow-up [28]. An epidemiological study, which included 7198 patients, showed that standardized incidence ratio (SIR) of gastric cancer patients diagnosed with gastric ulcer which underwent vagotomy was 1.5 after the first 10 years and reduced to zero after the second 10 years. It should be noticed that SIR of gastric cancer patients diagnosed with duodenal ulcer which underwent vagotomy was 1.3 both after the first and the second 10-years [8]. This suggests that vagotomy seemed to reduce the risk of gastric cancer.

Given that hypochlorhydria might be associated with increased concentration of carcinogenic *N*-nitroso compounds in the gastric juice [7], animal studies using vagotomy and *N*-methyl-*N*'-nitro-*N*-nitrosoguanidine in drinking water were conducted, and indeed vagotomy increased the number of adenocarcinomas formation in the stomach in these animal models [29, 30]. However, it should be noticed that the vagotomy performed in those studies was

without drainage procedures. Thus, the increased risk of gastric cancer upon vagotomy was most likely due to delayed gastric emptying, with increased exposure time of orally administered chemical carcinogens on the gastric mucosa in these animals. To study whether the vagus nerve has the direct trophic effect in the stomach, an experimental model of unilateral vagotomy has been used given that each (anterior or posterior) vagal trunk innervates only one half of the stomach. Consequently, denervation of one side of the stomach does not impair the overall functional capacity of the stomach, leaving gastric acid output, circulating gastrin levels and motility unchanged [31, 32]. Utilizing this approach, Håkanson *et al.* were able to demonstrate that the vagus nerve *per se* has the trophic effect on the gastric mucosa and that the vagus nerve is essential for the growth effect by PPI-induced hypergastrinemia on the gastric oxyntic mucosa in general and the enterochromaffin-like cells in particular [33, 34]. This was later confirmed by using gene knockout of muscarinic acetylcholine receptor type 3 (M3KO) mice [35].

1.4 Vagotomy Suppresses Gastric Tumorigenesis by Inhibiting WNT Signaling in Stem Cells

Given that the vagus nerve-mediated M3R signaling may potentiate a proliferative effect on gastric epithelium, it became again an interesting question whether the vagus nerve promotes gastric cancer development. Indeed, most of gastric cancers arise from lesser curvature of the stomach, where more pronounce vagus nerve ending is distributed [36, 37], suggesting the role of vagus nerve in tumor microenvironment. Very recently, the important role of the vagus nerve in gastric tumorigenesis has been investigated carefully in mouse models and in human patients [38]. Bilateral truncal vagotomy with pyloroplasty (VTPP) (Fig. 1) during the preneoplastic stage of tumorigenesis reduced the tumor incidence and size, and attenuated tumor cell proliferation specifically in the denervation portion of the stomach (after UVT) in the mouse models of gastric cancer, i.e., the genetically-induced mouse model (INS GAS) [39-

43], a chemically-induced (MNU) mouse model and a *Helicobacter pylori*-infected mouse model [44].

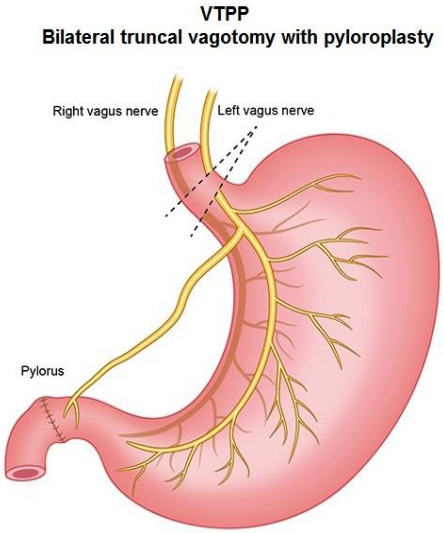

Figure 1: Drawing showing bilateral truncal vagotomy with pyloroplasty (VTPP).

More importantly, unilateral vagotomy (UVT) (Fig. 2), or local pharmacologic denervation via injection of botulinum toxin A in the gastric wall, similarly impaired preneoplastic growth, indicating the direct effect on tumor progression by vagus stimuli.

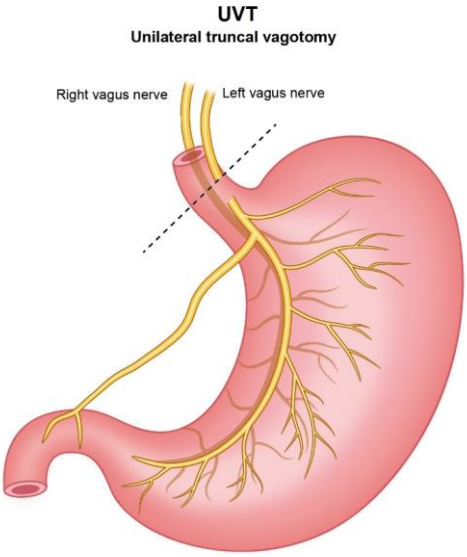

Figure 2: Drawing showing unilateral truncal vagotomy (UVT).

At later stages of gastric tumorigenesis, vagotomy, botulinum toxin A injection, or systemic treatment with darifenacin, an M3R antagonist, suppressed the cancer progression and augmented the anti-tumor effect of chemotherapy, leading to prolonged survival (Fig. 3).

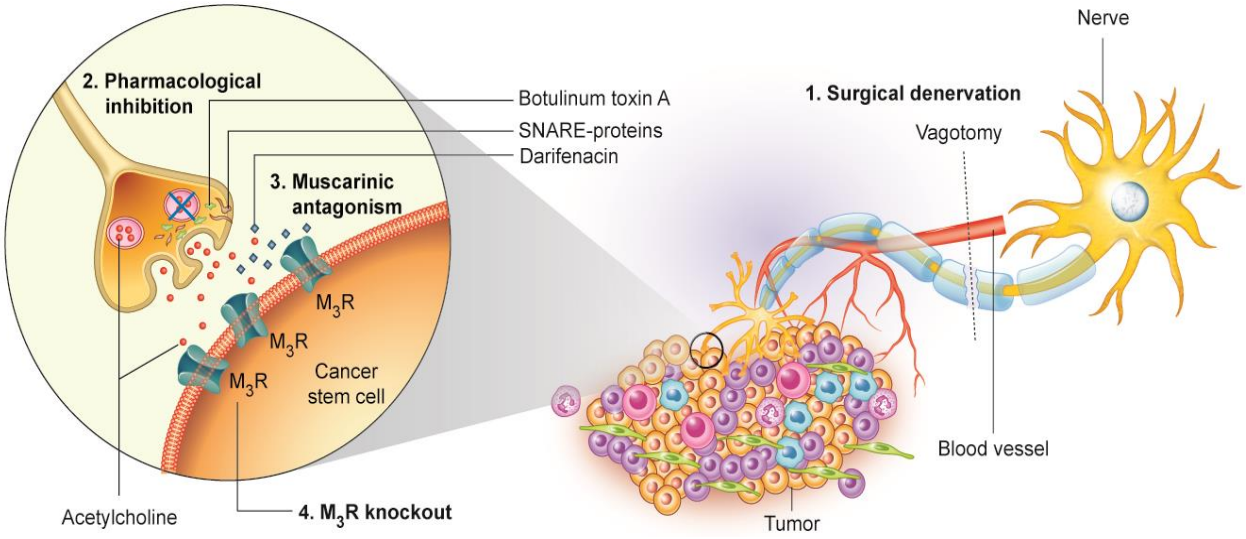

Figure 3: Drawing showing the important role of cholinergic signaling through the vagus nerve in tumorigenesis which have been demonstrated by four different approaches: 1. Surgical denervation by vagotomy; 2. Pharmacological inhibition by Botulinum toxin A which acts on SNARE-proteins; 3. Muscarinic receptor type 3 (M3R) antagonism on cancer stem cells by Darifenacin; and 4. M3R knockout.

In patients who had undergone unilateral vagotomy, gastric cancer did not occur in the vagotomized part of the stomach. Furthermore, the progression stage of gastric cancer was found to be significantly correlated with pronounced nerve supply [38]. Comprehensive gene expression analysis of gastric tumors with or without vagotomy showed that this denervation effect was mediated predominantly by inhibition of WNT signaling, which is a known major regulator of gastrointestinal stem cells and tumor formation. In earlier studies, it has been suggested that aberrant acetylcholine-M3R signaling causes transformability in part through WNT/β-catenin signaling activation [45, 46]. Consistent with those findings, vagotomy

inhibited the expansion of leucine-rich repeat containing G protein-couple receptor 5-positive ($Lgr5^+$) stem cells during carcinogenesis by suppressing WNT signaling in the stomach. In the stomach, the most abundant acetylcholine receptor is M3R, followed by low expression of M1R (muscarinic acetylcholine receptor 1) expression, and they are known to be expressed in parietal cells and chief cells [47]. Interestingly, $Lgr5^+$ stem cells specifically express high levels of M3R, and can be activated by acetylcholine and then activated $Lgr5^+$ cells promote epithelial proliferation. Thus, nerves play an important role in stem cell niche and tumor microenvironment, providing new insights into the cellular and molecular bases of tumorigenesis and pointing to the potential utility of anti-neurogenic therapies (Fig. 3).

TREATING CANCER BY GETTING ON ITS NERVES: FUTURE DIRECTIONS

Very recently, the so-called "nerve-cancer cell cross-talk" has been highlighted mainly based on the findings that targeting nerve fibers in prostate and gastric cancer can inhibit tumor growth and metastasis [48, 49, 38]. In addition, various signaling from nerves contributes to tissue and tumor stem cell niche in several organs [50-54]. Since local injection of botulinum toxin A has been successfully tested in animal models of gastric cancer [38] and prostate cancer (Gustavo Ayala: personal communication) and since botulinum toxin A seems to block the release of all kinds of neurotransmitters, two human clinical trials have been initiated in Norway and USA (https://clinicaltrials.gov/ct2/show/NCT01822210) (https://clinicaltrials.gov/ct2/show/NCT01520441).

The implication of denervation as therapeutic modalities for other cancers has not been reported yet, but could have great potential. For instance, the neural infiltration/invasion in the tumor is often observed and associated with poor prognosis e.g. in patients with adenocarcinoma of esophagogastric junction, cholangiocellular cancer or pancreatic cancer [55]. Neuronal activity promotes glioma growth [56], and blockade of the SNARE protein inhibits glioblastoma tumor growth [57].

The muscarinic receptors are also often found in the tumors of colon, pancreas, prostate, breast, lung, ovary, uterine cervix and skin [58]. Thus, treatment strategies targeting the neurotransmitter receptors and their downstream signaling pathways should be further explored [58, 59]. Recently, T-cell-based cancer immunotherapy shows a great promise in the treatment of patients with late-stage malignancies. Animal studies showed that electronic stimulation of the left cervical vagus nerve mobilized the spleen acetylcholine-synthesizing T cells, leading to reduced circulating tumor necrosis factor ß (TNF-ß) levels, which was most likely via a non-neural pathway between the vagus nerve and the spleen [60, 61]. However on the other hand, bilateral cervical vagotomy also attenuated the serum TNF-ß levels which were initially elevated in response to lipopolysaccharide treatment [62]. It will be of interest to explore the therapeutic concept of combining immunotherapy and anti-neurogenic therapy.

List of abbreviations

UVT = UnilateralTruncal Vagotomy

VTPP = Bilateral Truncal Vagotomy with Pyloroplasy

M3R = Muscarinic 3 Receptor

H2 blockers = Histamine type 2 Receptor Blockers

PPI = Proton Pump Inhibitor

H. pylori = *Helicobacter pylori*

SIR = Standardized incidence ratio

ELCs = Enterochromaffin-like cells

M3KO mice = Muscarinic Receptor 3 Knockout Mice

MNU = *N*-nitroso-*N*-methylurea

Lgr5+ stem cells = Leucine-rich Repeat containing G

Protein-couple Receptor 5-positive Stem Cells

TNF-ß = Tumor Necrosis Factor ß

Conflict of interest

The author(s) confirm that this article content has no conflict of interest.

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