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The effects of unilateral truncal vagotomy on gastric carcinogenesis in hypergastrinemic Japanese female cotton rats

Reidar Fossmark [a,b,*], Øystein F. Sørdal [a], Karin E. Bakkelund [a], Ivar Skjåk Nordrum [c,d], Helge Waldum [a,b]

[a] Department of Cancer Research and Molecular Medicine, Norwegian University of Science and Technology, Prinsesse Kristinas Gate 1, 7006 Trondheim, Norway
[b] Department of Gastroenterology and Hepatology, St. Olav's Hospital, Prinsesse Kristinas Gate 1, 7006 Trondheim, Norway
[c] Department of Laboratory Medicine, Children's and Women's Health, Faculty of Medicine, NTNU, P.O. Box 8905, Medisinsk teknisk forskningssenter, 7491 Trondheim, Norway
[d] Department of Pathology and Medical Genetics, St. Olav's Hospital, Erling Skjalgssonsgate 1, 7006 Trondheim, Norway

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ABSTRACT

The stomach is innervated by the vagal nerve. Several studies have demonstrated that the vagal nerve has a trophic effect on the rat oxyntic mucosa and that the trophic effect of hypergastrinemia is dependent on intact vagal innervation. The effect of vagal denervation on gastric carcinogenesis has been examined in *Mastomys natalensis* and hypergastrinemic transgenic INS-GAS mice, with no effect of unilateral vagotomy in Mastomys but an anti-carcinogenic effect in INS-GAS mice.

A proportion of female Japanese cotton rats develop spontaneous hypergastrinemia and ECL cell derived gastric carcinomas. In the current study we have examined the effects of unilateral anterior subdiaphragmatic vagotomy on gastric carcinogenesis. Female Japanese cotton rats were operated with unilateral anterior vagotomy or sham-operation at age 2 months and were terminated at age 10 months. Ten of fifteen animals operated by anterior vagotomy and 11 of 16 sham-operated developed hypergastrinemia. Vagotomy did not affect intragastric pH or serum gastrin. When comparing the anterior and posterior sides of the stomachs, vagotomy did not affect the occurrence of dysplasia or carcinoma development in the oxyntic mucosa. However, vagotomy resulted in lower stomach weight and reduced oxyntic mucosal thickness on the anterior side. Vagotomy also resulted in a reduction in volume density of chromogranin A positive cells in the oxyntic mucosa.

In conclusion, vagotomy reduced the trophic effects of hypergastrinemia on the ECL cell and oxyntic mucosa, but did not prevent gastric carcinogenesis in female Japanese cotton rats. The effects of vagotomy on gastric carcinogenesis in animal models are conflicting and further studies in patients should be done to clarify the clinically significant effects of vagotomy.

1. Introduction

The stomach is innervated by the vagal nerve with each vagal trunk innervating one side of the stomach. A trophic effect of the vagus nerve on the rat stomach was observed after unilateral vagotomy of the stomach, as the denervated side of the stomach displayed atrophy that was reflected in reduced weight and height of the oxyntic mucosa and a reduced density of argyrophil cells [1,2]. Furthermore, the trophic effect of hypergastrinemia induced by omeprazole was prevented by unilateral vagotomy and the number of enterochromaffin-like (ECL) cells was markedly reduced on the denervated side in rats [3]. Results from studies using tissue microdialysis suggest that the vagal nerve regulates histamine mobilization from rat stomach ECL cells by controlling their sensitivity to gastrin [4].

The role of vagal signalling has also been examined in genetically modified mice, and M3 muscarinic receptor knockout mice lack the trophic responses to hypergastrinemia [5]. More recently, gastric carcinogenesis in hypergastrinemic INS-GAS mice was found to be inhibited by unilateral truncal vagotomy [6]. However, *Mastomys natalensis* with loxtidine-induced hypergastrinemia develop ECL cell carcinoids, but vagotomy did not influence the development of carcinoids, dysplasia or ECL cell density in this animal model [7]. The role of the brain-gut axis in gastrointestinal carcinogenesis has not been fully elucidated and should be examined further in animal models of gastric carcinogenesis.

A proportion of female Japanese cotton rats develop hypergastrinemia secondary to hypo/anacidity which results in carcinomas in the oxyntic mucosa which we suggest develops from ECL cells [8]. Female cotton rats developing hypergastrinemia have increasing serum gastrin levels with onset from the age of 2 to 8 months [9]. More than 2 months of hypergastrinemia results in dysplasia in the oxyntic mucosa and more than 4 months of hypergastrinemia results in cancer in a large majority of the animals. Carcinoma development is gastrin dependent as it is prevented by a gastrin receptor antagonist [10] and is induced by hypergastrinemia due to dosing with the H2-blocker loxtidine [11] or

* Corresponding author at: Department of Gastroenterology and Hepatology, St. Olavs Hospital, Prinsesse Kristinas Gate 1, 7006 Trondheim, Norway. Tel.: +47 06800; fax: +47 72825734.
E-mail address: reidar.fossmark@ntnu.no (R. Fossmark).

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Table 1
Animal weight, intragastric pH, plasma gastrin and stomach weight in cotton rats operated with unilateral gastric vagotomy and controls (sham).

	Vagotomy-hypergastrinemic (n = 10)	Sham-hypergastrinemic (n = 11)	Vagotomy-normogastrinemic (n = 5)	Sham-normogastrinemic (n = 5)
Animal weight (g)	138.1 ± 4.56	140.1 ± 6.1	136.0 ± 10.7	153.3 ± 6.3
Intragastric pH	6.6 (5.1–7.4)*	6.1 (3.2–7.5)*	2.0 (1.7–2.1)	2.3 (1.9–2.6)
Plasma gastrin (pM)	584 ± 68	719 ± 130	16 ± 1.8	15 ± 3.1
Stomach weight (g)	2.8 ± 0.26**	3.7 ± 0.39	0.57 ± 0.05**	0.98 ± 0.03

* $p < 0.05$ compared to normogastrinemic controls.
** $p < 0.05$ compared to sham-operated hypergastrinemic or normogastrinemic animal.

partial corpectomy [12]. The carcinomas develop from a mucosa with hyperplasia of cells that are positive for neuroendocrine markers such as chromogranin A (CgA), pancreastatin and synaptophysin as well as histidine decarboxylase (HDC). The CgA and HDC mRNA expression is markedly increased in the oxyntic mucosa of hypergastrinemic animals [9,13]. A proportion of the carcinoma cells have similar immunohistochemical and morphological characteristics and we have previously argued that the tumours are ECL cell derived [9,11,13,14].

In the current study we have examined the effect of unilateral vagotomy on gastric carcinogenesis in female cotton rats.

2. Material and methods

2.1. Animals

Cotton rats were originally provided by Tanabe Seiyaku Co. Ltd, Toda, Japan in 1971 and maintained by random mating. In 1982 some of the animals had developed spontaneous gastric tumours and these animals were kept in a colony by sister/brother mating for more than 20 generations [15]. Cotton rats from this strain were housed in IVC cages with aspen woodchip bedding from B&K Universal Ltd., Hull, UK. Room temperature was 24 ± 1 °C with a relative humidity of 40–50% and a 12-h light/dark cycle. The B&K rat and mouse diet and tap water were provided *ad libitum*.

Sixteen female cotton rats, age 2 months, were operated by anterior truncal vagotomy on the lower oesophagus in isofluran inhalation anaesthesia. One animal died before termination of the study without blood or tissue samples. Sixteen animals were sham-operated and this operation consisted of laparotomy and gentle manipulation of the viscera. Antibiotics were not used. All animals received postoperative analgesia with buprenorphin 3 μg twice daily (Temgesic, Schering-Plough, New Jersey, USA) for 2 days. The study was approved by The Regional Committee for Animal Welfare Research Ethics and in accordance with the EU legislation for the protection of animals used for scientific purposes, Directive 2010/63/EU.

2.2. Termination and tissue collection

At termination of the study eight months after operation, blood was collected from the caval vein for measurement of plasma gastrin levels [16].

Intragastric pH was measured with the stomach *in situ* and the lowest pH registered before the animals were killed by exsanguination in isofluran anaesthesia. Stomach weight was recorded after the stomach was rinsed in NaCl 0.9%. Tissue samples from the anterior and posterior parts of the stomachs were collected as a continuous strip 5 mm from the border between the rumen and the oxyntic mucosa.

2.3. Histochemistry and Immunohistochemistry

Formalin fixed paraffin embedded specimens were cut into 4 μm thick sections and stained with haematoxylin and eosin. Sections from the oxyntic mucosa were evaluated by a pathologist blinded for operation status and histopathological changes on the anterior and posterior sides of the stomachs were recorded separately. The changes were categorized as hyperplasia, dysplasia and carcinomas [9].

Further sections were dewaxed and processed for immunohistochemistry. An antibody against the general neuroendocrine marker CgA was used as well as antibodies against more specific ECL cell markers such as vesicular monoamine transporter 2 (VMAT2) and HDC. Heat induced epitope retrieval by microwave cooking was then performed for 20 min in 10 mM Tris-EDTA buffer pH 9 (VMAT2 and HDC) and citrate buffer pH 6 (CgA). The primary antibodies were anti-CgA antibody 1:2000 dilution (#20086, Immunostar, Minnesota, USA), anti-VMAT2 1:2000 dilution (ab1767, Chemicon, Temecula, CA) and anti-HDC 1:8000 (Code B 260-1, Eurodiagnostica, Malmö. Sweden). The anti-CgA antibody was incubated at 4 °C overnight and the anti-VMAT2 and anti-HDC antibodies were applied at room temperature for 1 h. Antigen–antibody complexes were visualized by Envision-HRP kit (K5007, Dako, Denmark) followed by application of 3,3′ diaminobenzidine (DAB+) chromogen (K5007,

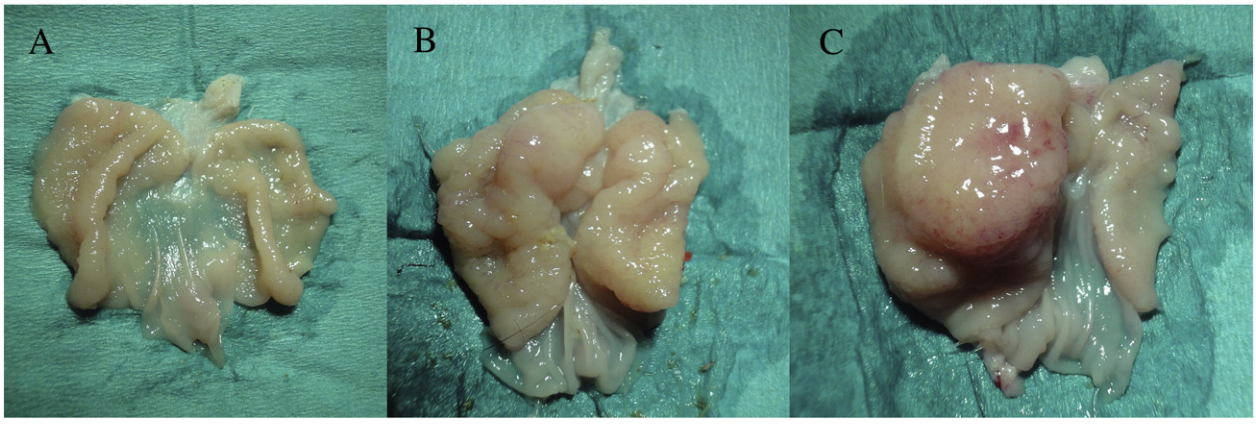

Fig. 1. Macroscopic appearance of the stomach of a normogastrinemic cotton rat (A), the stomach of a hypergastrinemic cotton rat which has general hypertrophic changes in the oxyntic mucosa (B) and a stomach with general hypertrophy and a macroscopic distinct tumour on the anterior side (C).