A Prospective Evaluation of the Effect of Chronic Proton Pump Inhibitor Use on Plasma Biomarker Levels in Humans

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Objective: Proton pump inhibitors (PPIs) are used primarily to treat gastroesophageal reflux disease. Proton pump inhibitor–induced achlorhydria increases circulating gastrin and chromogranin A (CGA). Chromogranin is a widely used biomarker for the diagnosis and follow-up for gut-based neuroendocrine tumors (NETs). Proton pump inhibitor–induced increases in CGA or gastrin may falsely suggest the presence of a NET when none exists. Pancreastatin, a fragment of CGA, is also commonly used to diagnose and follow NETs. We hypothesized that chronic PPI use would increase circulating plasma gastrin, CGA, and pancreastatin levels.

Methods: Thirty patients who used PPIs for 6 months or more (mean ± SD duration, 3.1 ± 2.5 years) and a separate control group of 30 patients who never used antacid medications were prospectively evaluated with plasma gastrin, CGA, and pancreastatin determinations.

Results: Chronic PPI use resulted in significant increases in CGA (15.1 ± 11 vs 131 ± 207 ng/mL; $P = 0.005$) and significant increases in gastrin (34.8 ± 22.3 vs 167.8 ± 136.2 pg/mL; $P = 0.001$) compared to controls. In contrast, pancreastatin level in nonusers and chronic PPI users were identical (81.6 ± 36.4 vs 89.4 ± 43.4 pg/mL; $P = 0.46$).

Conclusions: Pancreastatin levels do not change with chronic PPI use and normal pancreastatin levels may be used to distinguish between drug-induced changes in biomarkers and tumor–related increases in circulating biomarkers.

Key Words: chromogranin A, gastrin, pancreastatin, neuroendocrine tumor (NET)

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Proton pump inhibitors (PPIs) were introduced into clinical practice in the 1980s. Although the initial use of these compounds was limited to the control of hyperacidity associated with Zollinger Ellison syndrome (gastrinoma), their effectiveness rapidly amplified the clinical application of these compounds. The current over-the-counter availability of these agents and their efficacy in reducing symptoms of acid reflux makes their use almost universal in many populations. The ability of PPIs to induce gastric achlorhydria is well known, and the association between hypochlorhydria or achlorhydria and hypergastrinemia is also well established.[1]

Neuroendocrine tumors (NETs) are rare and produce symptoms that are often difficult to distinguish from the symptoms of more common conditions such as inflammatory bowel disease or irritable bowel syndrome. Serum biomarkers are commonly used to diagnose NETs in patients with common gastrointestinal complaints. Chromogranin A (CGA), a member of the granin family, has been widely accepted as the best biomarker for the diagnosis and subsequent follow-up of NETs.[2,3] Recently, pancreastatin, a small fragment of the CGA molecule, has been shown to be a sensitive and specific marker for NETs. The relative sizes of the CGA molecule and the pancreastatin fragment may provide significant increases in the sensitivity to detect small increases in neuroendocrine tumor volume.[4]

The chronic use of PPIs has been shown to induce both short-term and long-term elevations in CGA, making the differential diagnosis of NET versus PPI use difficult.[5] In addition, chronic PPI use has led some investigators to link chronic achlorhydria with the chronic gastrin-related stimulation of the enterochromaffin cells. These authors associate the subsequent development of enterochromaffin cell hyperplasia with the potential development of gastric carcinoids. Recent increases in the incidence of gut-based NETs such as gastric carcinoid has led some investigators to worry that chronic hypergastrinemia associated with widespread use of PPI therapy may be the culprit for this increase in NET incidence.[6]

Pancreastatin is a breakdown product of the large CGA molecule, and the cleavage of pancreastatin from CGA is controlled by the action of prohormone convertase 1. Recently, we and other groups have proposed that measurement of pancreastatin in patients with NETs may be more sensitive than the measurement of CGA levels. At least part of the increased sensitivity of pancreastatin versus CGA concentrations in the diagnosis of NETs is related to differences in plasma concentration (ng/mL for CGA vs. pg/mL for pancreastatin).[4]

Whereas it is well known that chronic PPI use increases circulating gastrin and CGA levels, the effect of chronic PPI use on circulating pancreastatin levels remains uninvestigated. Based on the stimulating effects of chronic PPI use on circulating plasma gastrin and CGA levels, we hypothesized that

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TABLE 1. Patient PPI Use

Patient Number	Sex	Age	Current PPI Used	PPI Dose	Total Duration of PPI Use
1	M	74	Omeprazole	20 mg/d	2.5 yrs
2	F	68	Esomeprazole (Nexium)	40 mg/d	5 yrs
3	M	66	Esomeprazole (Nexium)	40 mg/d	2 yrs
4	F	61	Omeprazole*	20 mg/d	5 yrs
5	F	74	Omeprazole	20 mg 1–2× per day	2 yrs
6	F	57	Esomeprazole (Nexium)†	40 mg 1–2× per day	5 yrs
7	M	53	Esomeprazole (Nexium)	40 mg/d	>5 yrs
8	M	75	Pantoprazole (Protonix)‡	40 mg/d	4 yrs
9	F	74	Omeprazole (Prilosec)	40 mg/d	>6 mos
10	F	35	Rabeprazole (Aciphex)	20 mg/d	>1 yr
11	F	49	Esomeprazole (Nexium)	40 mg/d	6 mos
12	F	74	Omeprazole (Prilosec)	20 mg 2× per day	2 yrs
13	F	68	Esomeprazole (Nexium)	40 mg/d	5 yrs
14	F	63	Esomeprazole (Nexium)	40 mg/d	1.5 yrs
15	F	85	Pantoprazole (Protonix)§	40 mg/d	>10 yrs
16	F	50	Omeprazole	20 mg/d	4 yrs
17	M	77	Pantoprazole (Protonix)	40 mg/d	9 mos
18	F	73	Pantoprazole (Protonix)	40 mg/d	10 mos
19	M	69	Rabeprazole (Aciphex)	20 mg/d	3 yrs
20	F	67	Omeprazole (Prilosec)	20 mg/d	10 yrs
21	M	55	Esomeprazole (Nexium)	40 mg/d	>3 yrs
22	F	82	Pantoprazole (Protonix)	40 mg/d	3 yrs
23	M	70	Esomeprazole (Nexium)	40 mg/d	2 yrs
24	F	68	Esomeprazole (Nexium)	40 mg/d	3 yrs
25	F	81	Omeprazole‖	20 mg/d	>5 yrs
26	F	28	Esomeprazole (Nexium)	40 mg/d	1 yr
27	F	64	Lansoprazole (Prevacid)¶	30 mg/d	21 mos
28	F	59	Esomeprazole (Nexium)	40 mg/d	6 mos
29	F	54	Omeprazole (Prilosec OTC)	20 mg/d	2 yrs
30	F	44	Esomeprazole (Nexium)	40 mg 2× per day	2 yrs

*Started on Prilosec, switched to trials of Protonix and Aciphex, changed to Nexium for 2 years, switched to the generic omeprazole for 6 months.
†Briefly switched to omeprazole, 20 mg/d; was changed back to Nexium.
‡Previously on Nexium, 40 mg/d for 3 years, and on Protonix for 1 year.
§Previously on Prilosec, switched to Nexium, currently on Protonix for 1 year.
‖Previously on Nexium, 40 mg/d for 5 years, switched to omeprazole for the past 3 months.
¶Previously on Nexium, 40 mg/d for 1 year; switched to Protonix, 40 mg/d for 6 months; currently on Provicid for 3 months.

chronic PPI use would also increase circulating pancreastatin levels.

MATERIALS AND METHODS

This study looks at the effects of PPI use in 2 different groups of patients. We prospectively studied 30 patients, all being treated for GERD, who used daily PPI therapy consistently for more than 6 months. We studied a separate group of 30 individuals who confirmed no history of PPI use and either none or rare use of H_2 antagonists or antacids in the past 3 years. These individuals served as the control for this study (Table 1). Each patient was asked to sign an informed consent approved by the institutional review boards (IRBs) for the Protection of Human Subjects of Ochsner Medical Center New Orleans, LA, and the Louisiana State University Health Sciences Center New Orleans, LA, on January 28, 2010. Patients who agreed to participate underwent a single blood draw using specialized collection tubes supplied by Inter Science Institute (Inglewood, Calif), the first of which occurred on February 24, 2010. These tubes contain a proprietary mixture of preservatives (Z-tubes). Immediately after the blood was collected, it was rapidly chilled and centrifuged at low speed (750 rpm). Plasma was separated from the specimen and immediately frozen until it was assayed for gastrin, CGA, and pancreastatin. Reference normal values used for this analysis included the following: gastrin level, less than 75 pg/mL; pancreastatin levels, 135 pg/mL; and CGA, less than 40 ng/mL. Each specimen was thawed only one time immediately before radioimmunoassay.[7] To control for the possibility of different gastrin, pancreastatin, or CGA assays, all assays were performed by Inter Science Institute

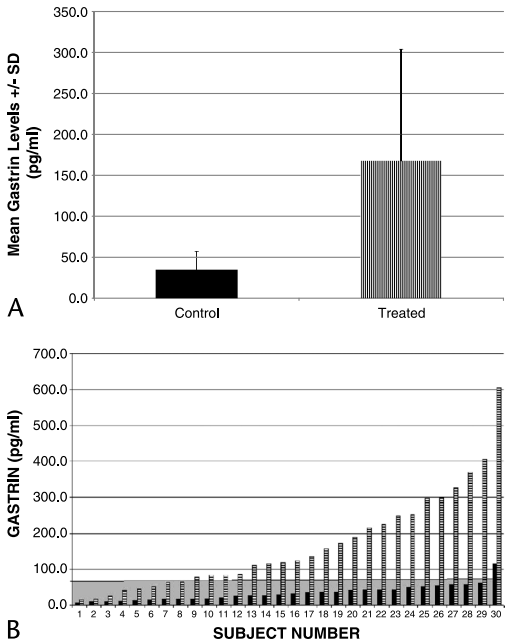

FIGURE 1. A, Mean ± SD gastrin levels in plasma from control individuals who had denied PPI/antacid use in the past 3 years (black bar) and in plasma from patients who chronically used PPIs (hatched bars). Differences between these means are statistically significant ($P = 0.001$). B, Individual gastrin levels in 30 chronic PPI users (hatched bars) and 30 control individuals (solid bars) who had denied PPI/antacid use in the past 3 years. The light gray shaded area represents normal gastrin levels.

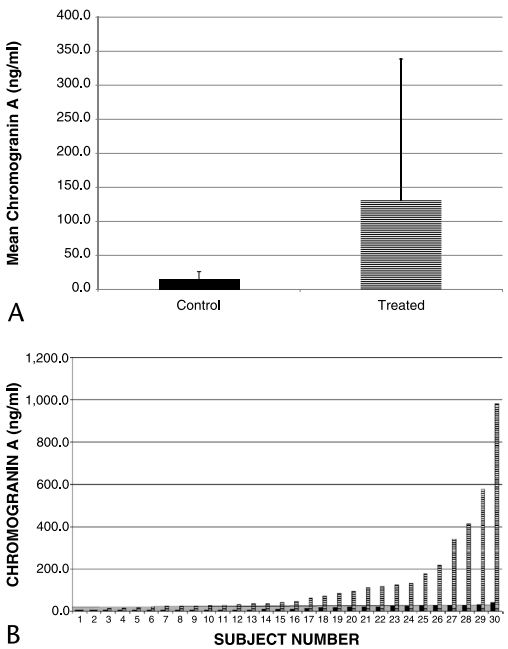

FIGURE 2. A, Mean ± SD CGA levels in plasma from control individuals who had denied PPI/antacid use in the past 3 years (black bar ± SD) and in plasma from patients who used PPIs chronically (hatched bars ± SD). Differences between these means are statistically significant ($P = 0.005$). B, Individual CGA levels in 30 chronic PPI users (hatched bars) and 30 control individuals (solid bars) who had denied PPI/antacid use in the past 3 years. The light gray shaded area represents normal CGA levels.

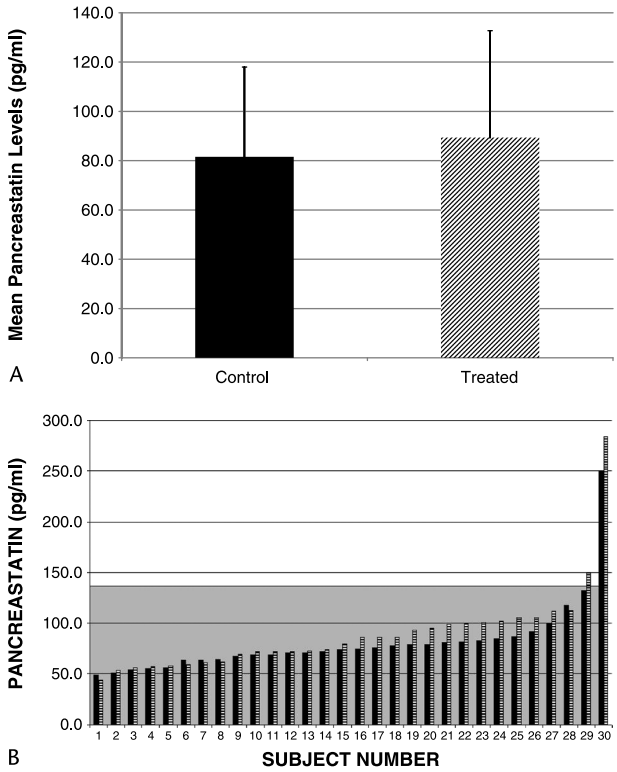

FIGURE 3. A, Mean ± SD pancreastatin levels in plasma from control individuals who had denied PPI/antacid use in the past 3 years (black bar ± SD) and in plasma from patients who used PPIs chronically (hatched bars). Differences between these means are not statistically significant ($P = 0.46$). B, This figure shows the individual pancreastatin levels in 30 chronic PPI users (hatched bars ± SD) and 30 control individuals (solid bars) who had denied PPI/antacid use in the past 3 years. The light gray shaded area represents normal levels.

(Inglewood, Calif). A paired *t* test was used to compare means of the control and PPI-treated groups. Statistical analysis was performed using the computer-based statistics program "A Primer of Biostatistics" (6th edition).[8] Results were considered significant at $P < 0.05$.

RESULTS

The control group had a male-female ratio of 15:15. The PPI group had a male-female ratio of 8:22. The mean ± SD age of the control group was 61 ± 12.6 years, and the mean ± SD age of the PPI group was 64 ± 13.5 years. Patients enrolled in this prospective study used PPIs over a long interval (mean ± SD duration, 3.1 ± 2.5 years). Chronic PPI use resulted in significant increases in CGA levels in the control group versus in the PPI user group (15.1 ± 11 vs 131 ± 207 ng/mL; $P = 0.005$; Fig. 1). Long-term PPI use also resulted in significant increases in circulating gastrin levels (34.8 ± 22.3 vs 167.8 ± 136.2 pg/mL; $P = 0.001$; Fig. 2). In contrast, pancreastatin levels in non-PPI users and in chronic PPI users were almost identical (81.6 ± 36.4 vs 89.4 ± 43.4 pg/mL; $P = 0.46$; Fig. 3).

TABLE 2. Patterns of Biomarker Elevation

	Gastrin	CGA	Pancreastatin	Gastric pH
Chronic PPI use	Elevated	Elevated	Normal	High
Type 1 gastric carcinoid	Elevated[9]	Elevated[9]*	Elevated*	High[10]
Pernicious anemia	Elevated†	Normal/elevated†	Normal†	High†
Zollinger-Ellison	Elevated[11]	Elevated[11]	Elevated[11]	Low[11]
Type 2 gastric carcinoid	Elevated[9,11]	Elevated[9,11]	Elevated[11]	Low[10]
Midgut NETs	Normal	Elevated[12]	Elevated[4]	Normal

*Based on the levels of patients with type 1 gastric carcinoid, seen in the Ochsner-Kenner Neuroendocrine Tumor Clinic; levels from patients' charts (n = 10).

†Based on the levels of patients with pernicious anemia, seen in the Ochsner-Kenner Neuroendocrine Tumor Clinic; levels from patients' charts (n = 5).

DISCUSSION

Use of PPIs has become widespread owing to the effectiveness of these medications and their over-the-counter availability. Patients with Zollinger-Ellison syndrome due to gastrinoma or type 2 gastric carcinoid have elevated gastric acid concentrations and elevated gastrin levels irrespective of their PPI use. These individuals also have increased circulating CGA levels. Conversely, patients with relative or absolute achlorhydria have increased circulating gastrin levels. Patients with pernicious anemia or type 1 gastric carcinoid have increased circulating gastrin levels and will also have elevated CGA levels. Patients with other gut-based NETs will commonly have elevated circulating CGA levels and normal gastrin levels. The concomitant use of PPIs in the presence of NETs confounds the difficulty in determining if the increase in plasma CGA or the increase in circulating gastrin is due to the NET or to the chronic PPI use (Table 2).

Chronic PPI use is associated with significantly elevated gastrin and CGA levels; the circulating pancreastatin concentrations remain within normal limits. A major limitation of this study is the lack of longitudinal data. We have no way of knowing if pancreastatin concentrations increased or decreased while remaining within the normal limits during the duration of this study.

Pancreastatin plays a large role in a variety of gut-based physiological processes. Furthermore, it can serve as a useful biomarker. If faced with a patient with elevated gastrin and CGA, the presence of a normal plasma pancreastatin level implies that the changes in biomarkers are from chronic PPI use or pernicious anemia rather than the presence of a NET. Conversely, elevated CGA levels or elevated gastrin levels in the face of elevated pancreastatin levels should prompt physicians to investigate the possibility of a NET. The cleavage of CGA into fragments such as pancreastatin by prohormone convertase I have traditionally been associated with NETs of the gastrointestinal tract. Our investigation may imply that prohormone convertase I fragmentation of CGA is associated with malignancy but not benign conditions; however, this has yet to be proven.

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REFERENCES

1. Laine L, Ahnen D, Mcclain C, et al. Review article: potential gastrointestinal effects of long-term acid suppression with proton pump inhibitors. *Aliment Pharmacol Ther*. 2000;14(6):651–668.
2. Woltering EA, Hilton RS, Zolfoghary CM, et al. Validation of serum versus plasma measurements of chromogranin A levels in patients with carcinoid tumors: lack of correlation between absolute chromogranin a levels and symptom frequency. *Pancreas*. 2006;33(3):250–254.
3. Vinik AI, Silva MP, Woltering EA, et al. Biochemical testing for neuroendocrine tumors (NETs). *Pancreas*. 2009;38(8):876–889.
4. O'Dorisio TM, Krutzik SR, Woltering EA, et al. Development of a highly sensitive and specific carboxy-terminal human pancreastatin assay to monitor neuroendocrine tumor behavior. *Pancreas*. 2010;39(5):611–616.
5. Giusti M, Sidoti M, Augeri C, et al. Effect of short-term treatment with low dosages of the proton-pump inhibitor omeprazole on serum chromogranin A levels in man. *Eur J Endocrinol*. 2004;150(3):299–303.
6. Winsett OE, Townsend CM Jr, Glass EJ, et al. Gastrin stimulates growth of colon cancer. *Surgery*. 1986;99(3):302–307.
7. Joseph S, Wang YZ, Boudreaux JP, et al. Neuroendocrine tumors: current recommendations for diagnosis and surgical management. *Endocrinol Metab Clin North Am*. 2010;40:205–231.
8. Glantz SA. The special case of two groups: the *t* test. In: *Primer of Biostatistics*. 6th ed. New York, NY: McGraw-Hill; 2005:73–125.
9. Hou W, Schubert ML. Treatment of gastric carcinoids. *Current Treatment Options in Gastroenterology*. 2007;10:123–133.
10. Burkitt MD, Pritchard DM. Review article: pathogenesis and management of gastric carcinoid tumors. *Aliment Pharmacol Ther*. 2006;24(9):1305–1320.
11. Gibril F, Jensen RT. Zollinger-Ellison syndrome revisited: diagnosis, biologic marker, associated inherited disorders, and acid hypersecretion. *Curr Gastroenterol Rep*. 2004;6:454–463.
12. DeHerder WW. Biochemistry of NETS. *Best Pract Res Clin Endocrinol Metab*. 2007;22:33–41.