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Pilot study of pyridostigmine in constipated patients with autonomic neuropathy

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Abstract

Background—The effects of cholinesterase inhibitors, which increase colonic motility in health, on chronic constipation are unknown. Our aims were to evaluate the efficacy of cholinesterase inhibitors for dysautonomia and chronic constipation and to assess whether acute effects could predict the long term response.

Methods—In this single-blind study, 10 patients with autonomic neuropathy and constipation were treated with placebo (2 weeks), followed by an escalating dose of pyridostigmine to the maximum tolerated dose (i.e., 180–540 mg daily) for 6 weeks. Symptoms and gastrointestinal transit were assessed at 2 and 8 weeks. The acute effects of neostigmine on colonic transit and motility were also assessed.

Results—At baseline, 4, 6, and 3 patients had delayed gastric, small intestinal, and colonic transit respectively. Pyridostigmine was well tolerated in most patients, improved symptoms in 4 patients, and accelerated the geometric center for colonic transit at 24 h by ≥ 0.7 unit in 3 patients. The effects of i.v. neostigmine on colonic transit and compliance predicted ($P < 0.05$) the effects of pyridostigmine on colonic transit.

Conclusions—Pyridostigmine improves colonic transit and symptoms in some patients with autonomic neuropathy and constipation. The motor response to neostigmine predicted the response to oral pyridostigmine.

Keywords

autonomic diseases; colon; constipation; gastrointestinal transit; pyridostigmine bromide

Introduction

Chronic constipation is a manifestation of diabetic autonomic neuropathy, pure autonomic failure, and multiple system atrophy [19,32,43]. Moreover, some patients with chronic idiopathic constipation without an underlying neurological disorder have a subclinical

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autonomic neuropathy, as documented by reduced sweating in response to cholinergic stimulation [1]. Therapeutic options for constipated patients with an autonomic neuropathy are limited [14]. By increasing the availability of acetylcholine, acetylcholinesterase inhibitors increase colonic motor activity in healthy subjects and reduce colonic distention in acute colonic pseudo-obstruction [23,33]. Neostigmine also increases motor activity in diabetics with constipation [4]. Case reports suggest that pyridostigmine improves constipation in Parkinson's disease [35] and autonomic neuropathy [31]. In a placebo-controlled trial of pyridostigmine (60 mg three times daily) in 126 patients with post-polio syndrome, pyridostigmine caused diarrhea in 55% of patients compared to 19% of patients on placebo [41]. However, the effects of pyridostigmine on symptoms and colonic transit in autonomic neuropathy and chronic constipation have not been systematically evaluated. Our hypothesis is that because extrinsic denervation is associated with denervation sensitivity in animal models [21,24,30], the response to cholinesterase inhibitors would be more pronounced in patients with autonomic neuropathy and constipation.

The aims of this study were first to assess the effects of pyridostigmine on symptoms, gastrointestinal, and colonic transit in constipated patients with an autonomic neuropathy; and second, to ascertain whether the acute effects of intravenous neostigmine on colonic transit and motility could predict the effects of oral pyridostigmine on colonic transit.

Methods

Overall design

This was a single-blind, placebo-controlled study comparing the effects of pyridostigmine and placebo on symptoms and gastrointestinal transit in 10 patients with autonomic neuropathy and constipation. Subjects were treated with placebo for 2 weeks then pyridostigmine for the next 6 weeks (Fig. 1). In addition to recording symptoms throughout this 8-week period, gastrointestinal transit was measured at 2 and at 8 weeks and colonic motility was assessed at 2 weeks. The acute effects of neostigmine on colonic motility and transit were also assessed at 2 weeks. This study was approved by the Mayo Clinic Institutional Review Board. All participants were recruited by public advertisement and signed informed consent.

Patients

Participants had an autonomic neuropathy with constipation, as defined by Rome II criteria, for at least 6 months prior to enrollment [40]. Structural and metabolic causes of constipation were excluded with appropriate laboratory tests, endoscopy, and/or imaging. Pelvic floor functions were evaluated by a clinical assessment (i.e., history and digital rectal examination) and anorectal testing (i.e., manometry and rectal balloon expulsion test). Patients with gastrointestinal or colonic resection, EKG abnormalities (i.e., second or third degree AV block, prolonged QT interval, or resting bradycardia [i.e., resting heart rate <45/minute]) or clinically significant cardiovascular, respiratory, or renal disease were not eligible to participate. With the exception of dietary fiber supplements and stool softeners, medications which could affect gastrointestinal motility were discontinued prior to the study. Stimulant laxatives (i.e., bisacodyl) and enemas were only permitted as "rescue agents", i.e., if subjects did not have a bowel movement for 3 consecutive days during the study. All females of child-bearing potential had to have a negative pregnancy test within 48 hours of the study.

Medications

Pyridostigmine is a quaternary ammonium compound, has variable oral bioavailability ranging from 11.5 to 18.9%, and is primarily excreted by the kidneys [2]. The prescribed dose of pyridostigmine for myasthenia gravis varies from 60 to 1,500 mg/day [18,42]. Because the optimum dose for increasing gastrointestinal motor activity is unknown, pyridostigmine was

begun at a dose of 60 mg orally three times daily and increased by 60 mg every 3 days, until subjects were satisfied with their bowel habits, or, were distressed by the side effects of pyridostigmine, up to a maximum dose of 180 mg three times daily. The pyridostigmine dose was reduced by 60 mg in patients who experienced intolerable side effects (e.g., abdominal cramping, lacrimation, or flushing). The maximum tolerated dose was maintained for the remainder of the 8-week treatment period.

For the first 2 weeks of the study, participants were treated with an escalating dose regimen of placebo capsules as described earlier. The consent form specified that patients would be treated either with placebo or pyridostigmine during the study, but did not specify the timeline for administering these medications. Medications were mailed to subjects at 2-week intervals.

Daily bowel diaries

As in previous studies, subjects recorded stool frequency, stool consistency (using the Bristol stool form scale), straining during defecation, and the sense of evacuation after defecation in validated daily bowel diaries [11,16,20]. The data were analyzed after averaging these symptoms for the entire 2-week placebo period and for 2 of the last 8 weeks during the pyridostigmine period in all subjects.

Weekly bowel diaries

In addition to daily diaries, patients rated symptom severity on a 5-point scale (0 = none, 1 = mild, 2 = moderate, 3 = severe, 4 = very severe) and medication efficacy on a 5-point scale (0 = none at all, 1 = a little bit, 2 = moderately, 3 = quite a bit, 4 = extremely) at 2 and 8 weeks. A change in severity by one or more grades was considered indicative of improvement or deterioration.

Scintigraphic transit: gastric emptying, small bowel, and colonic transit

Gastrointestinal transit was measured by standard, validated scintigraphic methods at 2 and 8 weeks [12,13]. Gastric and small bowel transit were measured by combining ^{99m}Tc -sulphur colloid with two eggs and then scrambling them, which were ingested with one slice of whole wheat bread and one glass of skim milk (300 kcal) [12,13]. A ^{111}In -labeled indium chloride (0.10 mCi) was mixed with a slurry of 5 mg activated charcoal in order to measure colonic transit [13]. The slurry was evaporated to dryness on a hot plate at 90°C, and the dried charcoal was placed into a size one gelatin capsule (Eli Lilly, Indianapolis, IN, USA) and coated with methacrylate (Eudragit S100, Degussa AG, Darmstadt, Germany), as in previous studies. A marker was placed on the patient's anterior superior iliac spine to map the location of the capsule in the colon. The capsule was given with a 3 oz. glass of water.

We obtained abdominal images every hour for the first 6 hours and at 8 and 24 hours. During the 2-week transit study, the effects of intravenous neostigmine (1 mg) on colonic transit were assessed after the 24 hour scan. In healthy subjects, intravenous neostigmine enhanced colonic propagation for up to 90 minutes after it was given [23]. Therefore, colonic transit was assessed at 15-minute intervals for 105 minutes after intravenous neostigmine in this study.

A variable region of interest program was used to measure transit as in previous studies [12, 13]. The proportion of ^{99m}Tc reaching the colon at 6 hour was used as a surrogate marker for small bowel transit. The primary summaries for comparison of transit profiles were: gastric emptying half-life time (t_{half}); colonic filling at 6 hour; colonic geometric centre (GC) at 24 hour; and ascending colon (AC) emptying t_{half} measurements based on geometric mean of counts in anterior and posterior AC regions of interest.

Data were analyzed as in previous studies. The GC is the weighted average of counts in the different colonic regions [ascending (AC), transverse (TC), descending (DC), rectosigmoid (RS)] and stool, respectively one-to-five. Thus, at any time, the proportion of colonic counts in each colonic region is multiplied by its weighting factor as follows: $(\%AC \times 1 + \%TC \times 2 + \%DC \times 3 + \%RS \times 4 + \%stool \times 5)/100 = GC$.

Colonic motility

After overnight fasting and oral lavage with 2–5 liters of polyethylene glycol 3350 and electrolyte solution (Golytely®, Abbott Laboratories, Chicago, IL, USA), a colonic barostat assembly was positioned using flexible endoscopy and fluoroscopy [7,29]. The multi-lumen polyethylene balloon barostat-manometric assembly was positioned with the barostat balloon in the descending (5 subjects) or, when limited by discomfort and/or anatomical factors, in the sigmoid colon (5 subjects). Tonic and phasic contractile activity of the colon were measured using an infinitely compliant 10 cm long balloon with a maximum volume of 600 cc (Hefty Baggies, Mobil Chemical Co., Pittsford, NY, USA) linked to an electronic barostat (Mayo Rigid Barostat, Mayo Foundation Engineering Department, Rochester, MN, USA). Fasting colonic tone, pressure-volume relationships (balloon inflation from 0 to 44 mmHg in 4 mmHg increments at 1 minute interval), the response to 1,000 kcal meal (750-mL, 53% fat), and to neostigmine (1 mg i.v.) were assessed in that order. Pressure-volume relationships were reassessed after neostigmine. Fasting colonic tone and postprandial contractile responses to a meal were recorded with the barostat balloon inflated to a fixed (i.e., “operating”) pressure as described previously [7,29]. Intra-balloon volumes and manometric pressure changes in response to wall contractions and relaxations were monitored continuously throughout the study. A pneumobelt was applied around the abdominal wall at the level of the lower costal margin to help exclude artifact during movement and coughing.

Colonic motor activity was summarized as described previously [7,29]. For the barostat balloon volume data, the baseline balloon volume representing colonic tone was separated from phasic volume deflections >10 mL from baseline volume. After averaging baseline balloon volumes for 30 minutes before and 60 minutes after the meal, the tonic response to a meal was analyzed as the proportionate volume change i.e., $[(\text{Post meal volume}/\text{pre-meal volume})/\text{Pre meal volume}] \times 100$. Compliance curves were approximated to a power exponential function summarized as P_{half} (the pressure at half maximum volume) [8]. For phasic pressure changes recorded by manometric sensors, data for high amplitude propagating contractions (HAPCs), i.e., contractions which were ≥ 75 mmHg and propagated caudally for 15 cm or more are included [23].

Gastroduodenal motility

Prompted by clinical features of intestinal dysmotility, gastroduodenal motility was also assessed in 5 patients with a multilumen ($n = 8$) assembly comprising five ports placed fluoroscopically across the antroduodenal junction and three ports in the duodenum and jejunum. The method has been described fully elsewhere [39]. Patients stopped taking any medication that might influence gastrointestinal motility at least 48 hours before the study. Pressure profiles were recorded for three hours during fasting and for two hours after a standardized meal. The gastrointestinal pressure profiles were used to diagnose or exclude intestinal pseudoobstruction.

Autonomic functions

Postganglionic sudomotor, cardiovascular, and adrenergic functions were evaluated [25]. Postganglionic sympathetic sudomotor axons were evaluated by the quantitative sudomotor axon reflex test (QSART) at four sites (forearm, proximal lateral leg, medial distal leg, and proximal foot) [26]. This test measures the sweat output in response to iontophoresis of

acetylcholine; the stimulus and response were recorded in different compartments of a multi-compartmental sweat cell. Cardiovascular functions were evaluated by heart-rate responses to deep breathing (HRDB) and the Valsalva ratio. HRDB was the heart-rate range with the subject supine and breathing at 6 breaths per minute. For the Valsalva maneuver, the subject was rested and recumbent and was asked to maintain a column of mercury at 40 mmHg for 15 seconds. The Valsalva ratio is the ratio of maximal-to-minimal heart rate [28]. Adrenergic function was evaluated by blood pressure (BP) and heart-rate responses, monitored continuously (Finapres monitor; Ohmeda, Englewood, CO, USA), to a Valsalva maneuver and head-up tilt. The results of the autonomic battery of tests were corrected for confounding effects of age and gender. The Composite Autonomic Severity Score (CASS) consists of three subscores: sudomotor (CASS-sudo; 0–3); cardiovascular (CASS-vag; 0–3); and adrenergic (CASS-adr; 0–4) [26]. The total score and subset scores provide an evaluation of the severity and distribution of autonomic failure.

Statistical analysis

In each subject, bowel symptoms from daily bowel diaries were collapsed across the first 2 weeks (i.e., placebo period) and for the last 2 (of the 8) weeks during treatment with pyridostigmine. Thereafter, paired *t* or sign tests were used to compare bowel symptoms assessed by daily diaries, measures of symptom severity and satisfaction at 2 and 8 weeks, and gastrointestinal and colonic transit at 2 (i.e., placebo) and 8 weeks (i.e., pyridostigmine). Separate linear regression models assessed whether the acute effects of neostigmine on colonic transit and compliance explained the effect of oral pyridostigmine on colonic transit; baseline colonic transit was a covariate in this analysis.

Results

Baseline characteristics

Thirteen patients with a primary diagnosis of autonomic neuropathy were screened for this study. Three patients with severe pelvic floor dysfunction were referred for pelvic floor retraining and excluded from the study. One patient with possible pelvic floor dysfunction could not undergo pelvic floor retraining and participated in the study. All 10 patients who were enrolled subsequently completed the study. In addition to autonomic neuropathy and constipation, 7 patients had lost ≥ 10 lb during their illness. Autonomic reflex testing revealed adrenergic dysfunction in 8 patients (adrenergic score = 2.1 ± 0.5), of moderate or worse severity in 6 patients. Seven patients had sudomotor dysfunction (score = 1.8 ± 0.4), which was moderate or severe in all patients. However, only 3 patients had cardiac vagal dysfunction (score = 0.4 ± 0.2), of moderate severity in 1 patient. Taken together, the composite autonomic severity score (CASS) score indicated mild (CASS < 4 ; $N = 3$ patients), moderate (CASS 4–6; $N = 5$ patients), or severe (CASS 7–10; $N = 2$ patients) autonomic failure.

At baseline, gastric emptying at 4 hours was normal in 5, delayed in 4, and accelerated in 1 patient (Table 1). Small intestinal transit was normal in 2, delayed in 6, and likely delayed but not measurable, because of marked intestinal dilatation and dilution of the isotope in 2 patients; 4 patients had clinical features and/or manometric evidence of intestinal and/or colonic pseudo-obstruction [9,39]. Colonic transit was normal in 6, delayed in 3, and not measurable, because the indium capsule did not reach the colon in 1 patient.

Effect of pyridostigmine on bowel habits and overall symptoms

The proportion of days on which subjects had one or more bowel movements increased ($P = 0.03$) from $55 \pm 8\%$ at 2 to $78 \pm 10\%$ at 8 weeks. However, pyridostigmine did not significantly affect the number of spontaneous complete bowel movements (1.8 ± 0.7 before versus 1.8 ± 0.8 after), overall stool consistency (2.3 ± 0.3 before versus 2.3 ± 0.3 after), straining during

defecation (2.1 ± 0.3 before versus 1.8 ± 0.4 after), or the sense of complete evacuation after defecation (2.0 ± 0.3 before versus 1.8 ± 0.4 after) as assessed by daily diaries. The effect of pyridostigmine on stool consistency (i.e., difference between scores at 2 and 8 weeks) was related ($r = 0.7$, $P = 0.04$) with straining, i.e., looser stools were associated with less straining.

Table 2 compares bowel symptoms at 2 and 8 weeks. At two weeks, 2 subjects each rated their constipation as absent, mild, moderate, or very severe and 1 patient rated it as severe. The severity of constipation score improved, albeit not significantly ($P = 0.25$ by sign test), from 2.0 ± 0.6 at 2 weeks to 1.4 ± 0.5 at 8 weeks. The severity of constipation improved by ≥ 1 grade on a 5-point scale in 3 patients, was unchanged in 5 patients, and worsened in 1 patient. The efficacy of treatment score improved ($P = 0.13$ by sign test) from 1.3 ± 0.5 at 2 to 2.6 ± 0.5 at 8 weeks. Thus, the reported efficacy of pyridostigmine was identical (5 patients) or better (4 patients, i.e., patients 6–9 in Table 1) than the reported efficacy for placebo.

Effect of pyridostigmine on gastrointestinal and colonic transit

Baseline transit data are summarized in Table 1. Gastrointestinal transit was studied in all 10 subjects at 2 weeks and in 9 subjects at 8 weeks. In one patient, the 8-week transit scans could not be retrieved for analysis. In addition, because ^{111}In did not reach the cecum, colonic transit could not be measured in 1 subject each at 2 and at 8 weeks. The 2-week transit study revealed delayed gastric emptying based on the 4 hour scan in 4 subjects, delayed small intestinal transit in 6 subjects, and delayed colonic transit in 3 subjects. Overall, gastric emptying, small intestinal ($33 \pm 14\%$ at 2 versus $41 \pm 11\%$ at 8 weeks), and colonic transit at 2 and 8 weeks were not significantly different (Figs. 2, 3). Neither gastrointestinal nor colonic transit improved in any patients with pseudo-obstruction. However, the GC_{24} for colonic transit increased by ≥ 1 in 2 patients (i.e., patients 3 and 8 in Table 1) and by 0.7 in 1 patient (i.e., patient # 6). Moreover, after adjusting for baseline colonic transit, the acute effect of neostigmine on colonic transit (i.e., the change between pre and 105 minutes post neostigmine) explained 75% of the variance in colonic transit at 8 weeks (i.e., after pyridostigmine, $P < 0.03$) (Fig. 4). The pattern and severity of autonomic dysfunctions and the dose of pyridostigmine were not different between responders and non-responders.

The relationships between pyridostigmine's effects on symptoms and colonic transit were borderline significant. Thus, the GC_{24} at 8 weeks (i.e., on pyridostigmine) was associated with higher stool consistency scores (i.e., looser stools, $r = 0.67$, $P = 0.07$) and also with a greater change in the sense of evacuation i.e., difference in sense of evacuation for pyridostigmine—placebo ($r = 0.68$, $P = 0.06$). However, the relationship between GC_{24} and straining during defecation was not significant.

Effects of neostigmine on colonic motility

At an operating pressure of 10.2 ± 0.8 mmHg (Mean $\pm$ SEM), fasting colonic balloon volume was 142 ± 27 mL and declined to 87 ± 26 mL during the 60-minute postprandial period, reflecting a 43% average postprandial augmentation of colonic tone. Four patients had colonic motor dysfunction, as evidenced by reduced fasting colonic tone (2 patients had balloon volumes of 265 and 276 mL at operating pressure) and/or a reduced colonic contractile response to a meal (3 patients) (Table 3). Three patients had a reduced tonic colonic contractile response to meal, i.e., the decline in postprandial volume was $<25\%$ of fasting volume. Neostigmine increased the Pr_{half} (i.e., pressure corresponding to half maximum volume) from 15.4 ± 1 mmHg to 19.8 ± 1.0 mmHg, indicating a shift to the right in the colonic pressure-volume relationship (Fig. 5). After adjusting for baseline colonic transit, the acute effect of neostigmine on colonic compliance explained 53% of the variance in colonic transit (i.e., GC_{24}) at 8 weeks (i.e., after pyridostigmine, $P = 0.04$). Manometric sensors revealed no propagated or high-

amplitude propagated contractions before neostigmine. Two subjects had HAPCs after neostigmine.

Side effects of pyridostigmine

Pyridostigmine was well tolerated at a dose of 442 mg [(240–540), mean (range)] daily. Five patients had no side effects, and 3 patients reported mild muscle twitching which was not bothersome; one of these patients also had mild nausea on pyridostigmine. One patient experienced sub-acute symptoms of muscarinic over-activity (diaphoresis, sweating, and abdominal cramps) that subsided in 2 hours. Thereafter, this patient completed the study, taking pyridostigmine at a lower dose of 420 mg, instead of 480 mg daily). Two subjects used bisacodyl during the first 2 weeks and throughout the study. Two subjects each used up to 4 phosphosoda enemas during the trial.

Discussion

Ten patients with an autonomic neuropathy and relatively severe gastrointestinal dysmotility manifested by clinical features and objective evidence of gastrointestinal, and in particular, colonic dysmotility were studied. In this pilot study, the cholinesterase inhibitor pyridostigmine was reasonably well tolerated, but did not significantly improve symptoms, gastrointestinal or colonic transit. However, pyridostigmine accelerated colonic transit in 3 patients. Two of these patients and an additional 2 patients reported that pyridostigmine improved symptoms more than placebo. Conversely, none of 4 patients with clinical and/or manometric features of chronic intestinal pseudo-obstruction associated with autonomic neuropathy responded to pyridostigmine. The sample size in this study is too small to determine whether the nature of the autonomic dysfunction influences the response to pyridostigmine.

Assessments of colonic tone revealed motor dysfunction in 4 patients, extending previous studies which observed reduced colonic phasic contractile responses to a meal in diabetes mellitus and reduced rectal sensation in patients with slow transit constipation and a small fiber neuropathy [5,22]. Neostigmine significantly increased the Pr_{half} , reflecting a rightward shift or increased tone, during the colonic pressure—volume relationship. In 5 patients, the Pr_{half} increased by 5 mm Hg or more which was the average change in Pr_{half} after neostigmine (0.75 or 1.5 mg i.v.) in a previous study of healthy subjects [23]. Neostigmine had more prominent effects on lower, rather than higher pressures, consistent with the effects of increased tone on pressure—volume relationships [23]. Moreover, neostigmine reduced compliance in 2 patients who had a reduced gastrocolonic contractile response to a meal, underscoring the utility of a pharmacological “challenge” for comprehensively assessing colonic motor functions and identifying inertia [3,5,6,38]. Indeed, the acute effects of neostigmine on colonic transit and separately on colonic motility predicted the effect of oral pyridostigmine on colonic transit. These observations suggest, for the first time, that the acute effects of a pharmacological agent on colonic motor functions predict the effects of oral therapy on motor endpoints (i.e., colonic transit) in patients with a gastrointestinal motility disorder. While it is conceivable that neostigmine accelerated colonic transit by inducing colonic HAPCs [23], only 2 patients had HAPCs after neostigmine during a colonic motility study. However, nonpropagating colonic contractions may also be associated with propagation of colonic contents [15]. Whether increased colonic tone per se can move colonic contents is unknown.

We opted for this study design because the syndrome (i.e., autonomic neuropathy with constipation) is relatively uncommon and recruitment is challenging. Moreover, our observations demonstrate considerable inter-patient variability in the type and severity of autonomic neuropathy and the extent and severity of gastrointestinal dysmotility. Because each patient served as his or her own control, this study design permitted within-patient comparisons, thereby reducing the impact of inter-patient variability on treatment effects. Because some

patients had very severe symptoms, it was not feasible to include a treatment-free washout period between placebo and pyridostigmine periods. Although we cannot exclude crossover effects, subjects were not informed that medications would be switched at 2 weeks into the study.

Our data suggest that a pyridostigmine dose of 360 mg daily or higher was well tolerated by 8 of 10 subjects. This dose was higher than the dose used to treat orthostatic hypotension in patients with autonomic neuropathy [34,36,37,44]. While stool consistency improved in 5 patients, abdominal discomfort worsened in 5 patients, perhaps reflecting a cholinergic side effect. Pyridostigmine dosing is complicated by erratic absorption and variable renal elimination. Indeed, there is up to a fivefold inter-individual and a twofold intra-individual variation in plasma concentrations after the same dose in healthy subjects [10]. The relationship between plasma concentrations of pyridostigmine and gastrointestinal effects has not been studied. However, the relationship between plasma concentrations and effects on peripheral neuromuscular function are not linear. Indeed, high plasma concentrations, often associated with doses exceeding 400 mg daily, may result in cholinergic overstimulation and impaired neuromuscular function [10].

In summary, these observations suggest that pyridostigmine may improve symptoms and accelerate colonic transit in a subset of patients with constipation and autonomic neuropathy. Based on these observations, a controlled study assessing the effects of pyridostigmine on symptoms and colonic transit in patients with an autonomic neuropathy and constipation is warranted, particularly since pyridostigmine also improves orthostatic intolerance, which is a common manifestation of autonomic neuropathy [36,37]. Because the inter-patient variability in baseline colonic transit (i.e., GC₂₄) was much higher (65%) than in healthy subjects (37%) [17], a parallel group study with 80% power to identify a clinically significant difference in GC₂₄ (i.e., 1 unit) after, versus before, treatment would require 29 subjects per group. For a study similar to this study, 13 subjects would be required. Excluding patients with intestinal pseudo-obstruction from the trial would reduce variability and also perhaps improve the likelihood of treatment success.

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Figure 1.

Experimental design. Subjects were treated with placebo for 2 weeks followed by pyridostigmine for the next 6 weeks. Symptoms, colonic transit, and colonic motility were assessed as shown

Figure 2.

Pyridostigmine did not have a significant effect on gastric emptying at 2 hours (**a**), or at 4 hours (**b**). The *shaded areas* represent the normal ranges

Figure 3.

Effects of cholinesterase inhibitors on colonic transit. During the baseline study (i.e., 2 weeks), intravenous neostigmine accelerated colonic transit in 2 subjects, shown by *dotted lines*. Oral pyridostigmine also accelerated colonic transit (i.e., GC₂₄ at 8 weeks) in both subjects compared to baseline. Pyridostigmine also accelerated colonic transit in another subject. Patient identifiers are identical to those in Table 1

Figure 4.

Effect of neostigmine and pyridostigmine on colonic transit. During the baseline (i.e., pre-treatment) scan at 24 hours (*left panel*), the isotope was in the ascending colon. After intravenous neostigmine (i.e., 105 minutes later), much of the isotope had been expelled in a bowel movement and the remainder was in the rectosigmoid colon. After oral pyridostigmine (*right panel*), the isotope was predominantly distributed in the rectosigmoid and, to a lesser extent, in the descending colon

Figure 5.

Effect of neostigmine on colonic motility in one patient. Observe that balloon volume declined, reflecting increased colonic tone, after neostigmine but not after a meal (1,000 kcal). Also observe that volumes during the “staircase” pressure—volume relationship were lower after than before neostigmine

Table 1

Baseline clinical characteristics

Number	Age, gender	Duration	Gastrointestinal transit			CASS	Non-GI features of autonomic neuropathy	Comments
			Stomach	Small intestine	Colon			
1	43, F	24 months	N	NA	N	3	Cholinergic autonomic neuropathy, neurogenic bladder	Antral hypomotility, normal N small bowel manometry
2	18, M	14 months	↑	↓	N	7	Acute panautonomic neuropathy, neurogenic bladder	Clinical intestinal pseudo-obstruction, 38 lb weight loss, enteral nutrition
3	66, M	15 months	N	↓	↓	4	Subacute autonomic cardiovascular adrenergic neuropathy, neurogenic bladder	Severe constipation and anorexia; 30 lb weight loss
4	55, M	15 months	N	N	N	4	Subacute autonomic cardiovascular adrenergic neuropathy, neurogenic bladder	Clinical and manometric intestinal pseudo-obstruction; 30 lb weight loss
5	67, F	5 years	↓	N	↓	8	Panautonomic neuropathy, neurogenic bladder	Manometric intestinal pseudo-obstruction
6	55, M	6 months	N	↓	N	4	Diabetes mellitus with adrenergic and sudomotor autonomic neuropathy	10 lb weight loss, pelvic floor dysfunction—insurance denied reimbursement for pelvic floor retraining
7	19, M	24 months	↓	NA	NA	4	Panautonomic neuropathy	Intestinal and colonic pseudo-obstruction 20 lb weight loss, on enteral and parenteral nutrition
8	64, M	3 months	↓	↓	↓	5	Acute idiopathic mononeuritis multiplex of undetermined etiology with adrenergic and sudomotor autonomic neuropathy	5 lb weight loss
9	41, M	13 months	N	↓	N	1	Autoimmune autonomic neuropathy with cardiovascular dysfunctions	70 lb weight loss, normal intestinal manometry
10	41, F	72 months	↓	↓	N	3	Postviral cholinergic autonomic neuropathy, Adie's pupil	65 lb weight loss,

NA intestinal or colonic transit could not be measured because of technical limitations, N normal, ↑ increased, ↓ decreased

Table 2

Effects of drugs on bowel symptoms assessed by weekly diaries

Symptom	Comparison of symptoms after pyridostigmine (i.e., 8 weeks) versus placebo (i.e., 2 weeks) ^a		
	Worse	Unchanged	Better
Abdominal discomfort	5	1	3
Abdominal bloating	2	6	1
Sense of incomplete evacuation	2	4	3
Hard stools	1	3	5
Straining during defecation	1	5	3

^aWorsening or improvement was defined as ≥ 1 point deterioration or improvement in symptom severity respectively on 5-point symptom severity scales for each symptom. Missing data for 1 subject who did not complete 8-week questionnaires

Table 3

Colonic motility findings

Parameter	Result	Number of patients with abnormal values
Fasting colonic volume (mL)	142 ± 27	2
Postprandial increase in colonic tone (%)	43 ± 10	3
Post-neostigmine change in colonic compliance (Pr _{half} , mmHg)	4.4 ± 0.6	4

Pr_{half} pressure corresponding to half maximum volume during the pressure-volume relationship. Neostigmine increased the Pr_{half}; reflecting lower compliance, or a stiffer colon