Dose-response effect of a β3-adrenergic receptor agonist, solabegron, on gastrointestinal transit, bowel function, and somatostatin levels in health

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Am J Physiol Gastrointest Liver Physiol 294: G1114–G1119, 2008. First published March 27, 2008; doi:10.1152/ajpgi.00051.2008.—β3-Adrenoceptors (β3-ARs) are expressed in the human gastrointestinal tract (3, 19). β3-AR is a member of the family of G-protein-coupled receptors that have been cloned from human, mouse, and rabbit and are expressed in adipocytes, heart, skeletal muscle, and smooth muscle of the gastrointestinal and urogenital systems. β3-AR expression is colocalized with choline acetyltransferase in a majority of the neurons in human colonic myenteric and submucosal plexus (12). The human selective β3-AR agonist, solabegron, inhibits cholinergic contractions and enhances release of somatostatin with no effect on carbachol-induced contractions in human isolated colon. A rodent selective β3-AR agonist inhibits castor oil-induced diarrhea in rats (12).

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Grudell ABM, Camilleri M, Jensen KL, Foxx-Orenstein AE, Burton DD, Ryks MD, Baxter KL, Cox DS, Dukes GE, Kelleher DL, Zinsmeister AR. Dose-response effect of a β3-adrenergic receptor agonist, solabegron, on gastrointestinal transit, bowel function, and somatostatin levels in health. Am J Physiol Gastrointest Liver Physiol 294: G1114–G1119, 2008. First published March 27, 2008; doi:10.1152/ajpgi.00051.2008.—β3-Adrenoceptors (β3-ARs) are expressed in the human gastrointestinal tract (3, 19). β3-AR is a member of the family of G-protein-coupled receptors that have been cloned from human, mouse, and rabbit and are expressed in adipocytes, heart, skeletal muscle, and smooth muscle of the gastrointestinal and urogenital systems. β3-AR expression is colocalized with choline acetyltransferase in a majority of the neurons in human colonic myenteric and submucosal plexus (12). The human selective β3-AR agonist, solabegron, inhibits cholinergic contractions and enhances release of somatostatin with no effect on carbachol-induced contractions in human isolated colon. A rodent selective β3-AR agonist inhibits castor oil-induced diarrhea in rats (12).

β3-AR agonist reduces the elevated tone and inhibits spontaneous contractions in the human isolated colon, but the exact site of action is not clear. Activation of β3-AR results in inhibition of cholinergic contractions, and enhanced release of somatostatin agonists of these receptors have been shown to inhibit spontaneous contractions of the human colon and relax precontracted colonic longitudinal and circular muscle (4, 16, 22). They also slow gastrointestinal transit in wild-type mice but have no effect in β3-AR knockout mice (18). In a rat model of diarrhea, the β3-AR agonist CL316243 decreased castor oil-induced fecal weight (12). On the other hand, β3-AR agonist does not alter carbachol-induced contractions in human isolated colon (12).

Visceral sensitivity is also controlled in part by adrenergic modulation. The nonselective β3-adrenergic receptor isoprotrenol leads to release of somatostatin. Somatostatin is released from enteroeocdocrine and neural elements in the gastrointestinal tract and is thought to act as an endogenous analgesic substance (25). Levasseur and colleagues (20) showed that a β3-adrenergic receptor agonist led to release of somatostatin from rat gastric antral cells, which was blocked with an antagonist. β3-AR agonist inhibited mustard oil-induced visceral pain via somatostatin receptor-2 activation (12).

Given the colocalization on cholinergic neurons, it would appear that a β3-adrenergic agonist might be proposed for treatment of D-IBS. Currently, loperamide and alosetron are the main pharmacological therapies available for patients with D-IBS (2). Although loperamide is available over the counter, alosetron is not prescribed extensively given its potential to cause ischemic colitis.
Our aims were to assess dose-related effects of solabegron on gastrointestinal and colonic transit, bowel function, and plasma somatostatin levels in healthy human volunteers and to characterize the pharmacokinetic profile of solabegron and its active metabolite following single and multiple dosing.

METHODS

**Trial design.** We performed a single-center, double-blind, randomized, placebo-controlled study to compare gastrointestinal transit between two different doses of solabegron (50 mg twice daily, 200 mg twice daily, n = 12 in each treatment group) and placebo (ClinicalTrials.gov Identifier: NCT00401479; GSK study no. B31106248). Allocation was concealed. Normal-weight participants were recruited, without restriction for gender, race, or ethnicity through public advertisement. All participants gave written, informed consent to participate in this study, which was approved by the Mayo Clinic Institutional Review Board.

**Study procedure.** Study participants were recruited by public advertisement and were enrolled in the study for 3 wk (including screen, 7 days of study medication, and follow-up). At the screening visit, subjects underwent a history and physical exam, laboratory work (chemistry, complete blood cell count, urinalysis), ECG, pregnancy test, were given a bowel habit diary to record their baseline bowel function using the Bristol Stool Scale for stool consistency rating (21). They returned for vital sign check on the next 6 days, participants took the study medication twice daily. bowel habit diary and receipt of the treatment bowel habit diary, in 7–14 days for repeat ECG, pregnancy test, were given a bowel habit diary to record their baseline bowel function using the Bristol Stool Scale for stool consistency rating (21) and completed the Bowel Disease Questionnaire (26). They returned in 7–14 days for repeat ECG, pregnancy test, return of their baseline bowel habit diary and receipt of the treatment bowel habit diary, receipt of study medication, and blood work (somatostatin levels). For the next 6 days, participants took the study medication twice daily. They returned for vital sign check on study day 2 and study day 5. On study days 6, 7, and 8 they completed the scintigraphic gastrointestinal transit test. Blood samples were also obtained for measurement of somatostatin and solabegron on study days 1 and 6 to determine both plasma concentrations and pharmacokinetic parameters, respectively. Seven to 14 days after the last dose of study medication, they returned for a final visit in which blood work, ECG, physical exam, and pregnancy test were repeated. The experimental design is summarized in Fig. 1.

**Solabegron.** Solabegron hydrochloride is a potent and selective in vitro agonist of human β3-AR activity compared with human β1- and β2-adrenergic activity and is not an antagonist at either β1- or β2-ARs. Solabegron is a biaryl phenethanolamine compound ([1-(1-biphenyl)-3-carboxylic acid, 3’-(2-(2-chlorophenyl)-6-hydroxyethyl)amino(ethyl)amino]-hydrochloride) of the class described by Uehling et al. (27). The primary metabolite of solabegron is the O-acetyl glucuronide metabolite solabegron; it has similar activity to solabegron as a selective β3-adrenergic agonist.

Solabegron is a β3-adrenergic agonist that has been shown to reduce nerve-stimulated colonic smooth muscle contractions and stimulate release of somatostatin, which has analgies properties, in human tissues. The pharmacokinetics of both solabegron (parent) and its active metabolite have been studied extensively in both single- and repeat-dose studies (unpublished data). Both parent and active metabolite are rapidly absorbed and depict a median time of maximum concentration (Tmax) of 1.5–3 h and 3–4 h, respectively, upon repeat dosing. Mean elimination half-life for parent compound ranges from 5 to 8 h and from 4.5 to 7.5 h for active metabolite. Both solabegron and active metabolite increase in a less than dose-proportional manner at doses ranging from 300 to 400 mg twice daily.

Prior to our trial, solabegron had been tested in 442 human subjects at doses ranging from 25 mg daily to 400 mg twice a day (unpublished data). At doses less than 200 mg per day, no clinically significant adverse events occurred; with a dose of 400 mg administered orally twice a day for 12 days, the most common adverse event was headache.

**Assessment of stool frequency and consistency.** During the study, patients completed a daily diary to record their bowel habits and to allow stool frequency, stool consistency, ease of passage, and sense of incomplete evacuation to be compared between the baseline and treatment periods.

Stool frequency was defined as the number of episodes of defecation recorded per day in the bowel habit diary; stool consistency was defined by the seven-point adjectival scale, which ranges from incontinence to requiring manual disimpaction (13); and sense of incomplete evacuation was defined by a yes-or-no answer to the question “Did you feel like you completely emptied your bowels?”

**Clinical laboratory tests.** Hematology, clinical chemistry, and urinalysis testing were performed at screen and follow-up visit or the early termination visit; other tests only at screening were serum concentration (Tmax) of 1.5–3 h and 3–4 h, respectively, upon repeat dosing. Mean elimination half-life for parent compound ranges from 5 to 8 h and from 4.5 to 7.5 h for active metabolite. Both solabegron and active metabolite increase in a less than dose-proportional manner at doses ranging from 300 to 400 mg twice daily.

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| Table 1. Demographics and posttreatment responses of the 3 treatment groups |
|--------------------------|--------------------------|--------------------------|
|                         | Placebo | Solabegron 50 mg | Solabegron 200 mg |
| Number (no. male)       | 11 (3)  | 13 (3)          | 13 (4)            |
| Age, yr                 | 37.4±3.0 | 34.8±2.9       | 38.0±3.1         |
| Body mass index, kg/m²  | 23.7±1.1 | 24.5±1.2       | 25.7±1.4         |
| Gastric emptying, 1/12, min | 142.7±13.4 | 139.2±7.9   | 137.6±6.0         |
| %Colonic filling at 6 h | 37.5±8.9 | 51.8±10.4     | 33.6±9.2         |
| Mean no. of stools/day  | 1.2±0.1 | 1.2±0.1        | 1.2±0.1          |
| Mean stool form score   | 3.6±0.2 | 3.7±0.3        | 3.3±0.2          |
| Mean ease of passage score | 3.94±0.03 | 3.95±0.07     | 3.79±0.08         |
| Mean proportion of bowel movements with incomplete evacuation | 0.09±0.03 | 0.17±0.06 | 0.17±0.06 |

Values are means ± SE. t1/2, Half time of elimination of the drug.

**Fig. 2.** Overall colonic transit by geometric center (GC) at different times; no significant differences between the active treatment groups and placebo. The GC at each time point was calculated by using the geometric mean transit times of all individuals. The mean value for the placebo group was calculated from the data, and the GC at each time point was calculated by using the geometric mean transit times of all individuals.
thyroid stimulating hormone and amylase. For female subjects of childbearing potential, a urine pregnancy test was conducted prior to administration of study medication on day 1 and within 48 h prior to exposure to any radiation.

Gastrointestinal transit measurement with scintigraphy. We have previously used the method (14) extensively and have demonstrated the reproducibility and performance to be expected of transit measurements obtained with scintigraphy. In this technique, a methacrylate-coated, delayed-release capsule containing 0.1 mCi of $^{111}$InCl$_3$ absorbed on activated charcoal particles is ingested. Two hours after ingestion of this capsule, two scrambled eggs labeled with $^{99m}$Tc-sulfur colloid are ingested with one slice of whole wheat bread and one glass of skim milk. Anterior and posterior gamma camera (Siemens, Diacam, Malvern, PA) images are then obtained at 0, 1, 2, 4, 6, 24, and 48 h after radioactive meal ingestion. The primary outcome variables include the percentage of radioisotope emptied from the stomach at 1, 2, and 4 h, the gastric half-emptying time from linear interpolation of the gastric residuals, the percentage of colonic filling at 6 h, and the colonic geometric center (GC) at 4, 8, 24, and 48 h.

Plasma somatostatin. Serial blood samples for somatostatin were collected prior to dosing (~5 min) and at 30, 60, 120, and 240 min after administration of study drug on both day 1 and day 6. Each sample (10 ml) was collected in a chilled Vacutainer (BD, Franklin Lakes, NJ) and centrifuged at ~4°C. Plasma was separated and stored at ~20°C for future analysis using radioimmunoassay at a commercial laboratory (Inter Science Institute, Inglewood, CA) using an in-house antibody. The limit of detection of the assay is ~1 pg/ml, and interassay reproducibility expressed as percent CV ranges from 9.3 to 15.7% for plasma somatostatin concentrations of 35 and 5.4 pg/ml, respectively.

Pharmacokinetics. Serial blood samples for solabegron and its active metabolite were collected prior to dosing and at 0.5, 1, 1.5, 2, 4, 6, and 8 h after administration of study drug on both day 1 and day 6. Blood samples (~4.0 ml each) for pharmacokinetic analysis of solabegron and its primary metabolite were collected in tubes containing lithium heparin additive. Samples were centrifuged in a refrigerated centrifuge (~4°C) at ~1,500 g for 10–15 min, and the resulting plasma was pipetted and placed in appropriately labeled polypropylene storage tubes [3.6 ml Nunc tube (Roskilde, DK-4000)]. Plasma was stored immediately at ~20°C or below until transported for analysis. All plasma samples were analyzed for solabegron and its primary active metabolite by the Department of Worldwide Bio-Analysis in Drug Metabolism and Pharmacokinetics of GlaxoSmithKline. Plasma concentrations of solabegron and active metabolite were measured by liquid chromatography-tandem mass spectroscopy (methodology on file at Drug Metabolism and Pharmacokinetics, GlaxoSmithKline Pharmaceuticals). The calibration curve range for parent compound and active metabolite was 1–1,000 ng/ml.

Statistical analysis. An intent-to-treat analysis using all randomized subjects was performed. Any missing data was imputed, using the overall mean across all subjects for each end point, with adjustment in the degrees of freedom as needed.

Primary and secondary end points (colonic GC at 8, 24, and 48 h, gastric emptying at 2 and 4 h, colonic filling at 6 h, ascending colonic transit time, overall stool frequency, consistency, ease of passage) were analyzed by one-way analysis of covariance for the three treatment groups. Gender was used as a covariate in analyzing effects of solabegron on gastrointestinal transit. Specific pairwise comparisons (each dose vs. placebo) were also conducted. Descriptive statistics (mean, standard deviation, minimum, median, and maximum) were calculated for somatostatin plasma concentrations and pharmacokinetic parameters for solabegron and its active metabolite using noncompartmental methods.

Study power. The study was powered to detect a clinically meaningful difference in transit parameters, specifically colonic transit at 24 h and gastric emptying at 2 h; data describing stool frequency, stool consistency, and ease of passage were assessed in a descriptive fashion. The study had 80% power (using a two-sample $z$-test at a two-sided $\alpha$ level of 0.05) to detect effect sizes for colonic transit GC 24 and ascending colonic half time of elimination of the drug ($t_{1/2}$) of 39 and 35%, respectively. The proposed study would detect changes in transit of a magnitude that would be of clinical significance in patients with constipation (that is, a change in GC of 1 unit) treated with colonic prokinetics (5, 9, 23) or of similar magnitude in patients with diarrhea given a 5-HT$_3$ antagonist such as alosetron (28).
RESULTS

Participant demographics and main results. Table 1 shows patient age and gender distributions as well as summary data. One participant (placebo group) had missing data for only the GC at 8 h, one subject in the 50 mg group had missing data on all end points, and one subject in the 200 mg group had missing data for only GC at 48 h. Thus we did an imputation for, at most, two subjects, depending on the specific end point.

Effect of solabegron on colonic transit. There was no effect of treatment on overall colonic transit (Fig. 2) or on ascending colon emptying time (Fig. 3).

Effect of solabegron on bowel function. There was no effect of treatment on bowel function (Table 1).

Effect on plasma somatostatin. Figure 4 summarizes the plasma somatostatin concentrations observed at different times on day 1 and day 6. Note that there are no differences in the plasma levels in response to the 50 or 200 mg dose of solabegron.

Pharmacokinetics. As depicted in Table 2, following single and repeat oral dosing of solabegron at doses of 50 and 200 mg, solabegron was rapidly absorbed with median T_{max} values ranging from 1 to 3 h. Solabegron was also rapidly eliminated with mean T_{1/2} values ranging from 1.63 to 2.33 h. Mean area under the curve over time [AUC_{(0-\infty)}] and maximum concentration (C_{max}) values increased with the increase in dose from 50 to 200 mg in a less than dose-proportional manner. Small to moderate between-subject variability [24–40% coefficient of variation (CV)] was associated with the solabegron pharmacokinetic parameters. In addition, when subjects were administered solabegron at both dose levels, the results showed, as indicated in Table 3, that the active metabolite had a median T_{max} ranging from 2.96 to 4 h. Like the parent compound, the active metabolite was also rapidly eliminated with mean T_{1/2} values ranging from 2.34 to 3.28 h. Finally, mean AUC_{(0-\infty)} and C_{max} values increased with the increase in solabegron dose from 50 to 200 mg in a less than dose-proportional manner.

Safety. Solabegron was generally well tolerated. Twenty-two of the 36 subjects experienced at least one adverse event. The most common adverse events (>10%) reported were headache and various gastrointestinal complaints. There was one adverse event of headache reported as severe; all others were mild or moderate. Mean blood pressure and heart rate changes and outlier analyses were similar across both solabegron doses and placebo. There were no clinically significant changes in clinical chemistries or hematology.

DISCUSSION

This pharmacodynamic study in healthy volunteers did not detect a difference in the gastrointestinal or colonic transit in response to two specific doses of solabegron. Similarly, no significant changes were observed for bowel function by using descriptive analyses. The study was powered to detect a 35–39% change in colonic transit summaries, and clearly this was not achieved (see Figs. 2 and 3) in healthy human volunteers. This study did not detect stimulated release somatostatin in response to two doses of solabegron. The pharmacokinetic results of both solabegron (parent) and active metabolite are consistent with similar doses in previous phase I studies. Given the pharmacokinetic results of both solabegron and active metabolite, it is also apparent not only that subjects received study drug, but that solabegron exposures after both single and twice-daily dosing achieved expected levels. These results are informative in relation to the potential application of this pharmacological approach in patients with IBS.

The study had a number of strengths including the design and the validated measures of colonic transit by scintigraphy, which have been previously demonstrated to have defined performance characteristics that were accounted for in the sample size calculations. Significant alterations in these transit measurements in response to candidate drugs also have been predictive of efficacy (e.g., alosetron, renzapride, tegaserod, prucalopride, neurotrophins, lubiprostone, linaclotide) or lack of efficacy (e.g., piboserod) in phase IIB or phase III
clinical trials of agents that have well-defined motor effects. Therefore, using this approach to determine whether solabegron would appreciably alter colonic transit has construct validity.

A possible explanation for the failure to show a pharmacological effect on colonic transit or bowel function is that β3-AR agonists did not inhibit carbachol-induced colonic contractions in experimental animals. In IBS patients, the postprandial aggravation of symptoms may be driven by cholinergic input that may not be impeded, according to the experimental studies with carbachol. On the other hand, β3-AR agonists alter colonic tone in experimental animals, suggesting that this motor effect may alter colonic compliance, and this may also contribute to its effects on visceral sensation. The effects of solabegron on visceral sensitivity, compliance, tone, and phasic and tonic responses to meal ingestion in IBS patients, as well as efficacy in phase II and phase III trials are therefore eagerly awaited. This is particularly relevant given the observations regarding the expression of β3-AR (12) in myenteric and submucosal neurons (including cholinergic neurons) in mammalian and rodent intestine, and the evidence that β3-AR modulation alters human colonic muscle contractility (16, 17, 22), colonic tone, and compliance in dogs (15).

The failure to detect increases in circulating plasma somatostatin in response to solabegron is not directly supportive of the hypothesis of a somatostatin-linked visceral analgesic effect of solabegron. However, this result on circulating plasma somatostatin concentrations does not discount the possibility of a local release of somatostatin by neurons in the submucosal and myenteric plexus or other nonneuronal cells such as immune cells and endocrine cells in the mucosa in response to solabegron and a consequent local effect on visceral hypersensitivity.

The safety of solabegron, as evidenced by adverse events, laboratory analyses, and vital signs, is similar to previous healthy volunteer studies at similar doses.

The limitations of this study are the relatively small sample size and the conduct of the study in healthy subjects rather than in patients. The former is a limitation, but it was guided by a power statement that suggests the lack of effect of solabegron on colonic transit does not represent a type II statistical error. The latter limitation has to be addressed by further studies in patients.

In conclusion, at the doses tested in healthy volunteers, the β3-AR agonist, solabegron, does not significantly alter gastrointestinal or colonic transit, bowel function, or plasma somatostatin concentrations. Solabegron and active metabolite exposures (AUC and C_{max}) at both 50 and 200 mg twice daily were consistent with pharmacokinetics at similar doses in previous phase I studies. Solabegron was generally well tolerated with few adverse events, and, specifically, there was no associated bowel dysfunction. Thus, although the first evaluation of the potential of modulating β3-AR mechanisms in healthy human volunteers suggests there is no significant effect on transit, further studies are required to evaluate the role of β3-AR modulation on colonic compliance, tone, and sensation in humans and on symptoms in patients with irritable bowel syndrome.

REFERENCES


