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A double-blind, randomized, dose response study testing the pharmacological efficacy of synthetic porcine secretin

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SUMMARY

Background: Biologically derived porcine secretin has been used as a diagnostic agent in clinical gastrointestinal practice for many years. Pure synthetic porcine secretin is now available for investigational clinical use.

Aim: To compare the pharmacology of synthetic porcine secretin and biologically derived porcine secretin in healthy volunteers.

Methods: Secretin stimulation tests were performed in 12 volunteer subjects in a double-blind, randomized, Latin square crossover design study comparing three doses of synthetic porcine secretin (0.05, 0.2, and 0.4 μg/kg) with a standard dose of biologically derived porcine secretin (1 CU/kg). Duodenal aspirates were analysed for total volume and for bicarbonate concentration. Total bicarbonate output was calculated.

Results: Twelve subjects completed four dosing regimens. A multiple comparison test was used to compare dosing regimens. The 0.2 and 0.4 μg/kg doses of synthetic porcine secretin were not different from the 1 CU/kg dose of biologically derived porcine secretin for volume, bicarbonate concentration and total output from 0 to 60 min. Only one patient had an adverse event, which was mild, transient flushing after the 0.2 and 0.4 μg/kg doses of synthetic porcine secretin and after the 1 CU/kg dose of biologically derived porcine secretin.

Conclusions: Synthetic porcine secretin has identical pharmacologic effects to biologically derived porcine secretin in normal subjects. Both drugs were safe and well-tolerated. This study validates synthetic porcine secretin as a substitute for biologically derived porcine secretin.

BACKGROUND

Secretin, a gastrointestinal peptide hormone produced by duodenal mucosal cells, stimulates the pancreas to produce bicarbonate-rich pancreatic juice. Jorpes & Mutt originally extracted secretin from porcine duodenum in 1961 and later defined the standard unit of activity for secretin.[1, 2]

In clinical practice, biologically derived porcine secretin (Secretin-Ferring, Ferring Pharmaceuticals, Inc., Tarrytown, NY) has been used in the United States since 1981 for the following indications: (i) to assess pancreatic exocrine function; (ii) to diagnose gastrinoma (Zollinger–Ellison syndrome); (iii) to obtain desquamated pancreatic cells for cytopathological examination; and (iv) to identify the minor papilla during endoscopic retrograde cholangiopancreatography through stimulation of pancreatic secretion.

Pancreatic exocrine function is typically assessed via secretin stimulation testing. There are published data documenting expected secretory response for normals and for patients with pancreatic disease using this diagnostic modality.[3, 4] Whilst secretin stimulation testing is an accepted means to evaluate pancreatic

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function, there can be variation in pancreatic response in normal subjects and in patients with pancreatic disease. Much of this variation may be due to the heterogeneity of extracted porcine secretin and batch variability, and inter-operator differences when performing the test. Despite these inadequacies, there is evidence to support that a properly performed secretin stimulation test will yield reliable results.[4, 5]

Secretin is used as a diagnostic agent for gastrinoma (Zollinger–Ellison syndrome), a gastrin-secreting tumour. Secretin administered intravenously produces an exaggerated gastrin response in patients with gastrinoma. This response is the basis for using secretin as a diagnostic test in the evaluation of patients in whom gastrinoma is suspected.[6–10]

Cytopathological evaluation of pancreatic juice collected from patients with suspected pancreatic disease has proven useful in the diagnosis of pancreatic carcinoma.[11, 12] Following the administration of intravenous secretin, pancreatic juice can be collected from the pancreas during cholangiopancreatography or from the duodenum during pancreatic stimulation testing.[12]

Secretin is used to help identify the location of the minor papilla in patients with pancreas divisum. In these patients, the main pancreatic duct drains through the minor papilla instead of its normal opening, the major papilla. This is due to failure of the dorsal and ventral ducts to fuse during embryogenesis. During cholangiopancreatography, it may be impossible to identify the location of the minor papilla and/or its orifice in order to cannulate it and inject dye to provide for radiographic visualization of the main pancreatic duct. In this event, secretin can be injected, often causing a gush of pancreatic juice through the minor papilla, making the orifice of the pancreatic duct easily visible to the endoscopist.[13]

Despite the clinical utility of secretin as a diagnostic agent in clinical gastrointestinal practice, the biologically derived porcine secretin product has been discontinued and is no longer available in the United States. As a result, synthetic porcine secretin (ChiRhoClin, Inc., Silver Spring, MD) has been developed and is now available in the United States for investigational use. Synthetic porcine secretin is the identical 27 amino acid peptide found in biological porcine secretin, but it is almost completely pure (> 96% vs. 60%) and is synthesized and manufactured under good manufacturing practices (GMP) rather than extracted from porcine intestine. The synthetic product has three major advantages over the extracted product: (i) synthetic secretin is homogeneous and compositionally consistent and should produce a consistent pharmacologic effect; (ii) synthetic secretin is a defined chemical entity and is free of animal pathogens that may be present in the porcine intestine; and (iii) synthetic secretin can be produced in quantities large enough to provide a reliable supply to the medical community for clinical use.

Extracted porcine secretin and synthetic porcine secretin have not been compared in clinical trials to date. In addition, the original pharmacological response study in 1972 was obtained on an unapproved formulation of secretin and the clinical procedures and science have substantially improved since this initial study.[6] Therefore, this study was conducted to establish the pharmacological activity and dose–response of synthetic porcine secretin in normal healthy subjects undergoing evaluation of exocrine pancreas function—an approved, well-documented diagnostic use of secretin. These data document the safety and pharmacological equivalence of synthetic porcine secretin relative to biologically derived porcine secretin and identify the appropriate dose(s) to use diagnostically.

We hypothesized that: (i) synthetic porcine secretin will demonstrate equivalent pharmacological activity to extracted porcine secretin in normal healthy volunteers as measured by pancreatic secretory response; and that (ii) synthetic porcine secretin will be safe and well-tolerated by the study population at the doses tested.

PATIENTS AND METHODS

We tested our hypotheses in a single centre, prospective, double-blind, randomized, Latin square crossover design study comparing three doses of synthetic porcine secretin with a standard dose of biological porcine secretin. The Latin square crossover design permitted each subject to serve as his or her own control, which minimized variability and increased the precision of the statistical analysis.

Healthy, non-smoking, volunteers between the ages of 18 and 65 years, within 20% of ideal body weight and without a medical history of pancreatitis, vagotomy, inflammatory bowel disease, or liver disease, were included. Females were required to be of non-childbearing potential. No alcohol was permitted within 72 h of each test and no anticholinergics within 1 month of screening. Other than hormone replacement or oral

contraceptives, no prior or concomitant medication was permitted within 72 h of enrolment.

The volunteer subjects were randomly assigned to a specified sequence of four dosing regimens. The volunteers underwent standard secretin stimulation testing on four separate occasions, with at least 24 h between each test. On each occasion they received one of the following doses:

- Dose A: synthetic porcine secretin, 0.05 μg/kg;
- Dose B: synthetic porcine secretin, 0.2 μg/kg;
- Dose C: synthetic porcine secretin, 0.4 μg/kg;
- Dose D: extracted porcine secretin, 1 CU/kg.

Previous cat bioassays demonstrated that synthetic porcine secretin and biologically derived porcine secretin have identical pharmacology (unpublished data, on file at ChiRhoClin, Inc. and FDA). The quantitative pharmacological relationship based on relative purities of the two products and cat bioassay results established 0.2 μg of synthetic porcine secretin as equivalent to 1 CU of biologically derived porcine secretin, the standard clinical dose per kg used in the secretin pancreatic stimulation test. In order to evaluate the dose response of synthetic porcine secretin, we chose to compare 0.05, 0.2, and 0.4 μg/kg of synthetic porcine secretin to 1 CU/kg of biologically derived porcine secretin.

An independent pharmacist dispensed the drug in syringes identical in appearance. No modifications of the commercial product were made. Biologically derived porcine secretin was reconstituted according to the directions in the package insert, and administered at a dose of 1 CU/kg (0.1 mL/kg). Synthetic porcine secretin was reconstituted with sterile normal saline to achieve an injection volume of 0.1 mL/kg for each of the three dose levels (0.05, 0.2, and 0.4 μg/kg).

Secretin stimulation testing was performed in standard fashion. A double lumen tube was placed with fluoroscopic guidance into the stomach and duodenum. Baseline duodenal aspirates were collected for two 10-min periods prior to dosing. After dosing, two 10-min collections and two 20-min collections were obtained, consecutively.

Ethical considerations

This study was approved by the Institutional Review Board at Duke University Medical Center and the General Clinical Research Center's (GCRC) Scientific Advisory Committee. All subjects gave informed consent to participate in the study.

Sample size calculation

Using a one-way repeated measures analysis of variance with four levels, participation of 12 individuals was required to detect a 20% change from the reference product with a $P = 0.05$ and 80% power. The bicarbonate concentration in mEq/L was utilized to determine the number of subjects required for the study.

Statistical analysis

The analyses of the comparative pharmacodynamic effects of synthetic porcine secretin and biologically derived porcine secretin are presented in two ways: unadjusted for basal (pre-secretin) exocrine pancreas function; and adjusted for basal exocrine pancreas function by subtracting the mean baseline values. Whilst not clinically applicable, this analytical approach allows one to compare the pharmacodynamic activities of the two exogenous secretin preparations in normal subjects, whilst taking into account basal pancreatic function and endogenous secretin effect.

The effect of the four doses on pancreatic juice volume, bicarbonate concentration, and total bicarbonate output were compared using the general linear model (GLM) procedure from Statistical Analysis System (SAS Institute, Cary, NC). Multiple comparisons and regression procedures with and without baseline adjustments were utilized to compare the doses.

RESULTS

A total of 15 subjects were enrolled. Two subjects were unable to swallow the dreiling tube and were withdrawn from the study before receiving any study drug. They were not included in the analyses. One subject completed the first test, but did not tolerate the intubation easily and elected to withdraw from the study. This subject completed protocol specified safety follow-up assessments and was analysed for safety, but excluded from the primary pharmacological analyses. Twelve subjects completed all four tests and were fully analysed for pharmacological and safety measures.

Demographics

The analysed study population consisted of four males and nine females with a mean age of 28.9 years (range 22–46 years). Racial representation included 11 Caucasian, one Asian, and one Native American. Medical history showed no current clinically significant medical problems.

Pharmacological analyses

Samples were analysed for total volume and for bicarbonate concentration. Total bicarbonate output was calculated. Results by dose, volume, bicarbonate concentration and total bicarbonate output are shown in (Table 1) for each 10-min collection and for the 60-min post-dose collection combined.

Table 2 displays the results comparing the four groups for baseline unadjusted and adjusted 60-min post-dose collections combined. The three escalating doses of synthetic porcine secretin produced a linear dose response for total volume of pancreatic juice but similar results for bicarbonate concentration from 0 to 60 min. The 0.2 μg/kg and 0.4 μg/kg doses of synthetic porcine secretin were not different from the 1 CU/kg dose of biologically derived porcine secretin for volume of juice, bicarbonate concentration, and total bicarbonate output from 0 to 60 min. Baseline adjusted bicarbonate concentration from 0 to 60 min was not statistically different for any dose.

When the 0.2 μg/kg dose of synthetic porcine secretin and the 1 CU/kg dose of biologically derived porcine secretin were compared, significant differences were found between the baseline unadjusted bicarbonate concentration in the 10–20 min collection ($P = 0.01$) and the baseline unadjusted bicarbonate concentration in the 0–60 min collection ($P = 0.0496$).

Safety

Physical examination, clinical laboratory assessments, and electrocardiograms (EKGs) were all within normal limits or unchanged from baseline. Three adverse events were observed during this study. All occurred in the same subject who experienced transient mild flushing of the face and extremities for approximately 5 min after receiving synthetic porcine secretin at the 0.2 and 0.4 μg/kg dose levels and after receiving biologically derived porcine secretin at the 1 CU/kg dose level. This

Table 1. Exocrine pancreas response to synthetic and biologically derived porcine secretins

		Collection period (min)						
Dose	Mean ± s.d.	−20 to −10	−10 to 0	0 to 10	10 to 20	20 to 40	40 to 60	0 to 60 Combine
Synthetic 0.05	Vol. (mL)	27.2 ± 17.0	19.7 ± 11.6	43.0 ± 21.9	35.9 ± 14.6	49.6 ± 21.7	41.3 ± 26.5	169.8 ± 54.1
	HCO_3 (mEq/L)	2.8 ± 7.6	3.4 ± 11.8	59.6 ± 33.2	104.7 ± 19.0	106.1 ± 15.7	89.3 ± 16.6	92.5 ± 16.7
	HCO_3 (mEq)	0.09 ± 0.25	0.10 ± 0.33	3.01 ± 2.35	3.69 ± 1.49	5.26 ± 2.45	3.69 ± 2.45	15.65 ± 5.87
Synthetic 0.2	Vol. (mL)	19.9 ± 19.6	9.8 ± 8.9	49.9 ± 21.6	40.5 ± 25.0	77.8 ± 39.2	67.2 ± 40.3	235.5 ± 92.2
	HCO_3 (mEq/L)	3.3 ± 8.1	5.3 ± 11.3	64.9 ± 24.1	91.2 ± 21.0	104.9 ± 6.3	100.6 ± 12.5	94.5 ± 7.4
	HCO_3 (mEq)	0.02 ± 0.05	0.05 ± 0.11	3.49 ± 1.99	3.67 ± 2.54	8.25 ± 4.25	6.75 ± 4.01	22.16 ± 9.12
Synthetic 0.4	Vol. (mL)	17.7 ± 16.5	12.8 ± 11.1	46.1 ± 30.4	38.9 ± 23.0	83.3 ± 32.7	100.3 ± 27.7	268.6 ± 67.5
	HCO_3 (mEq/L)	2.6 ± 5.2	3.9 ± 7.0	56.5 ± 32.7	93.6 ± 13.3	101.1 ± 12.0	98.4 ± 14.2	91.5 ± 13.8
	HCO_3 (mEq)	0.09 ± 0.20	0.04 ± 0.07	3.21 ± 2.48	3.86 ± 2.54	8.32 ± 3.10	9.75 ± 2.51	25.14 ± 7.74
Biological 1 CU	Vol. (mL)	21.8 ± 25.3	11.8 ± 9.0	45.2 ± 17.6	47.7 ± 20.8	76.0 ± 37.5	76.0 ± 44.5	244.8 ± 101.7
	HCO_3 (mEq/L)	5.3 ± 8.8	9.0 ± 10.7	76.0 ± 11.0	102.7 ± 6.8	106.6 ± 6.9	103.3 ± 9.8	99.7 ± 3.6
	HCO_3 (mEq)	0.13 ± 0.20	0.10 ± 0.15	3.52 ± 1.64	4.92 ± 2.24	8.00 ± 3.73	7.62 ± 4.09	24.05 ± 9.93

Table 2. Mean pancreatic stimulation results for 60 min interval—comparison of four dosing regimens

	60 min interval			60 min interval baseline adjusted*	
Dose	Volume mL	HCO_3 mEq	HCO_3 mEq/L	HCO_3 mEq/L	HCO_3 mEq
sPS 0.05	169.8	92.5	15.65	89.4	15.2
sPS 0.2	235.5	94.5	22.4	90.2	21.4
sPS 0.4	268.6	91.5	24.9	88.3	23.9
bPS 1 CU/kg	244.8	99.7	24.3	92.6	22.7
Probability	0.0001	0.1517	0.0006	0.7915	0.0009

sPs, synthetic porcine secretin; bPS, biologically derived porcine secretin.
*Mean baseline values subtracted.

subject did not have flushing with the lowest dose of synthetic porcine secretin (0.05 μg/kg). The three episodes of flushing were not associated with any changes in vital signs or other clinical symptoms. Each episode was classified as probably related to study drug and resolved spontaneously.

DISCUSSION

Synthetic porcine secretin is a pure, laboratory-synthesized version of biologically derived porcine secretin with the identical 27 amino acid sequence and structure. Biologically derived secretin carried the theoretical risk of transmitting animal pathogens and is no longer available in the United States. Synthetic secretin avoids both of these problems.

This study suggests that in normal healthy subjects, the 0.2 μg/kg dose of synthetic porcine secretin and the 1 CU/kg dose of biologically derived porcine secretin (the recommended dose for evaluating the exocrine pancreas) produced a statistically equivalent and numerically similar physiologic response of the pancreas as measured by volume, bicarbonate concentration, and total bicarbonate output. Over the dose range studied for synthetic porcine secretin, there was a modest dose response for volume and total bicarbonate output, but similar results for bicarbonate concentration. This suggests that synthetic porcine secretin is quite potent and that the standard clinical dose of 1 CU/kg or 0.2 μg/kg produces near maximal stimulation of the exocrine pancreas, on a plateau of the dose–response curve. In addition, total bicarbonate outputs for the entire 60 min collection were not different for the 0.2 μg/kg and 0.4 μg/kg doses of synthetic porcine secretin and the 1 CU/kg dose of biologically derived porcine secretin. However, output was significantly lower for the 0.05 μg/kg of synthetic porcine secretin, reflecting the impact of lower pancreatic juice volume. Both products at their respective doses appear to be equivalent for evaluation of exocrine pancreas function.

In this study, both synthetic porcine secretin and biologically derived porcine secretin were safe and well-tolerated at all doses tested. The only adverse event was transient flushing without changes in vital signs in one subject.

Based on the pharmacodynamic equivalence of synthetic porcine secretin and biologically derived porcine secretin in normals, and on the reliance of the diagnostic test on the same pharmacological effect, synthetic porcine secretin is likely to be an acceptable alternative to biologically derived porcine secretin as a diagnostic agent for chronic pancreatitis. A subsequent study comparing 1 CU/kg of biologically derived porcine secretin and 0.2 μg/kg of synthetic porcine secretin in pancreatic function testing in patients with chronic pancreatitis found that the synthetic porcine secretin was 100% accurate in diagnosing pancreatic insufficiency when compared to biologically derived porcine secretin.[14]

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REFERENCES

1 Jorpes E, Mutt V. On the biological activity and amino acid composition of secretin. Acta Chem Scand 1961; 15: 1790–1.
2 Jorpes E, Mutt V. On the biological activity of secretin. Acta Physiol Scand 1966; 66: 316–25.

3 Arvanitakis C, Cooke AR. Diagnostic tests of exocrine pancreatic function and disease. Gastroenterology 1978; 74: 932–48.
4 Gutierrez LV, Baron JHA. Comparison of Boots and GIH secretin as stimuli of pancreatic secretion in human subjects with of without chronic pancreatitis. Gut 1972; 13: 721–5.
5 Lagerlof HO, Schutz HB, Holmer S. A secretin test with high doses of secretin and correction for incomplete recovery of duodenal juice. Gastroenterology 1967; 52: 67–77.
6 Hansky J, Soveny C, Korman MG. Effect of secretin on serum gastrin as measured by immunoassay. Gastroenterology 1971; 61: 62–8.
7 Thompson JC, Reeder DD, Bunchman HH, Becker HD, Brandt EN. Effect of secretin circulating gastrin. Ann Surg 1972; 176: 384–93.
8 Deveney CW, Deveny KS, Jaffe BM, Jones RS, Way LW. Use of calcium and secretin in the diagnosis of gastrinoma (Zollinger–Ellison). Ann Intern Med 1977; 87: 680–6.
9 Lamers CBH, Van Tongeren JHM. Comparative study of the value of the calcium, secretin, and meal stimulated increase in serum gastrin to the diagnosis of the Zollinger–Ellison syndrome. Gut 1976; 18: 128–35.
10 Mihas AA, Hieschowitz BT, Gibson RG. Calcium and Secretin as provocative stimuli in the Zollinger–Ellison Syndrome. Digestion 1978; 17: 1–10.
11 Hatfield ARW, Smithies A, Wilkins R, Levi AJ. Assessment of endoscopic retrograde cholangiopancreatography (ERCP) and pure pancreatic juice cytology in patients with pancreatic disease. Gut 1976; 17: 14–21.
12 Naccarato R, Farini R, Matarazzo R, *et al.* Cytology of duodenal aspirate collected during secretin-pancreozymin test. An aid in the earlier diagnosis of pancreatic carcinoma. Ital J Gastroenterology 1978; 10: 14–7.
13 O'Connor KW, Lehman GA. An improved technique for accessory papilla cannulation in pancreas divisum. Gastrointest Endosc 1985; 31: 13–17.
14 Somogyi L, Cintron M, Toskes PP. Synthetic porcine secretin compared to biologically derived porcine secretin in pancreatic function testing. Gastroenterology 1999; 116: A1169.