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A randomized controlled crossover study comparing synthetic porcine and human secretins with biologically derived porcine secretin to diagnose Zollinger–Ellison Syndrome

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SUMMARY

Background: Although biologically-derived porcine secretin is approved for the diagnosis of Zollinger–Ellison Syndrome, it is no longer available in the United States. Pure human and porcine secretins have now been synthesized and new drug applications have been filed with the Federal Drug Administration (FDA).

Methods: In the current study we compared secretin testing results in six confirmed Zollinger–Ellison Syndrome patients using the biologically-derived product and both synthetic products (human and porcine) in a three-way, randomized, single-blind Latin-squares crossover study.

Results: Using the FDA-approved criterion for positive secretin testing (i.e. a serum gastrin concentration increase of > 110 pg/mL), there was complete agreement between all three agents for all patients. With the more stringent NIH criterion (i.e. a serum gastrin concentration increase of > 200 pg/mL), positive results persisted in five out of six, six out of six and four out of six patients using biologically-derived secretin, synthetic porcine secretin, and synthetic human secretin, respectively (six out of six, six out of six and four out of six if a positive test was defined as a 50% increase in serum gastrin concentration). The time to peak serum gastrin concentration after secretin injection occurred within 15 min in all studies (in 94% by 10 min and in 77% by 5 min). Three-way comparisons of serum gastrin concentrations showed a single statistically significant difference (the change from baseline at 15 min between synthetic human and synthetic porcine secretin, $P = 0.0274$). Statistically significant changes from baseline occurred at 1, 2 and 5 min for biologically-derived porcine secretin and at 2 and 5 min for both synthetic porcine and synthetic human secretin, in keeping with the expected time curve for positive tests. All three agents were well-tolerated.

Conclusions: These data suggest that either synthetic secretin product, when released onto the United States market, can be used to confirm Zollinger–Ellison Syndrome.

INTRODUCTION

Zollinger–Ellison Syndrome is characterized by a gastrin-releasing neuroendocrine tumour usually located in the pancreas or proximal duodenum, gastric acid hypersecretion, and severe, often fulminant, peptic ulceration.[1] The diagnosis of Zollinger–Ellison Syndrome is generally made by identifying inappropriate hypergastrinemia (an elevated serum gastrin in the presence of excessive gastric acid output) in conjunction with a compatible clinical scenario, such as the presence of peptic ulceration, chronic diarrhoea, or manifestations of multiple endocrine neoplasia type I.[1, 2]

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However, other conditions such as the retained gastric antrum syndrome, gastric outlet obstruction, antral G cell hyperplasia, antral-predominant *Helicobacter pylori* gastritis, massive intestinal resection, and chronic renal failure may also cause inappropriate hypergastrinemia.[1–3]

Most authorities would agree that patients with fasting serum gastrin concentrations greater than 1000 pg/mL in the presence of an elevated gastric acid output have either Zollinger–Ellison Syndrome or the retained gastric antrum syndrome.[2, 3] These conditions can generally be distinguished easily by whether or not the patient has undergone previous gastric surgery, and if so, by a sodium pertechnetate scan.[4] However, most patients with Zollinger–Ellison Syndrome present with hypergastrinemia in a range below 1000 pg/mL, and under these conditions, it may be difficult (without a confirmed tissue diagnosis) to distinguish inappropriate hypergastrinemia due to Zollinger–Ellison Syndrome from inappropriate hypergastrinemia due to other causes. In such situations, provocative tests such as the meal stimulation test, the calcium infusion test, or the secretin stimulation test have all been developed.[5–7]

The secretin test has long been felt to be the provocative test of choice because of its ease of administration, overall safety and accuracy.[1–3, 7] Secretin is a gastrointestinal peptide hormone consisting of 27 amino acids.[8] It is normally released from duodenal S-cells in response to a reduction in lumenal pH resulting from acid stimulation in response to food ingestion.[8] Secretin has a short half life in the blood (less than 5 min).[8] Its primary pharmacological action is on the exocrine pancreas where it binds to specific secretin receptors on pancreatic acinar cells leading to an increase in the volume and bicarbonate content of pancreatic juice.[8] This phenomenon has been exploited diagnostically for the secretin stimulation test to identify exocrine pancreatic insufficiency in which exogenous secretin is administered and the pancreatic juice bicarbonate concentration is measured. A bicarbonate concentration of < 70 mEq/L is felt to be diagnostic of pancreatic insufficiency after a 1-clinical unit/kg (CU/kg) intravenous injection of secretin.[8] Normally, exocrine pancreatic juice has a neutralizing effect on the duodenal contents, resulting in an increase in local pH which in turn inhibits the ongoing release of secretin from the duodenum (feedback inhibition).[8] However, secretin receptors are also found on a variety of other neuroendocrine cell types including gastrinoma cells. Gastrinoma cells respond to secretin by releasing their hormonal product (gastrin). This forms the basis of the secretin provocative test for Zollinger–Ellison Syndrome because antral G-cells (which also contain secretin receptors) have a less pronounced response to exogenous secretin administration.[1–3, 7]

While there is general agreement regarding the need to obtain provocative testing for patients suspected of having Zollinger–Ellison Syndrome, there has been much controversy regarding the definition of a positive secretin test.[1–3, 6–10] Using secretin biologically derived from porcine duodenum (formerly marketed by Ferring Pharmaceuticals, Tarrytown, NY), FDA approval was granted in 1981 for a 2-CU/kg body weight bolus injection of secretin with a positive test being defined as an increase in serum gastrin concentration of greater than 110 pg/mL following the injection.[11] The dose was expressed in terms of biological activity, not weight, because the biologically-derived preparation was impure. However, this criterion for a positive test has been questioned and two alternative criteria have been proposed. These include either greater than a 50% increase or greater than a 200-pg/mL increase in serum gastrin concentration following secretin injection.[7, 10] The latter definition (i.e. an increase of greater than 200 pg/mL), while being the most stringent, was shown to be the best potential cut-off after careful evaluation in a large single-site comparative trial at the National Institutes of Health.[7] On the other hand, a recent meta-analysis of studies that included only patients with a confirmed tissue diagnosis of gastrinoma suggested that the initial threshold of 110 pg/mL may be more appropriate.[12]

ChiRhoClin Inc. (Silver Spring, MD) recently synthesized a pure porcine secretin. The synthetic porcine secretin has an amino acid sequence identical to the biologically-derived product, but it is almost completely pure (greater than 96% vs. 60%). ChiRhoClin Inc. has also synthesized a pure human secretin which is identical in sequence and structure to natural human secretin. As illustrated in Figure 1, human and porcine secretins are both 27 amino acid peptides but they differ at amino acid positions 15 and 16.[8] The quantitative pharmacological relationships of the three secretins were determined in the validated cat bioassay used for release of the biologically-derived porcine secretin product. This assay, which is a measure of secretin's stimulatory effects on the exocrine pancreas, established that 0.2 μg of either of the two synthetic products is

Synthetic Human Secretin

H-His-Ser-Asp-Gly-Thr-Phe-Thr-Ser-Glu-
Leu-Ser-Arg-Leu-Arg-**Glu-Gly**-Ala-Arg-
Leu-Gln-Arg-Leu-Leu-Gln-Gly-Leu-Val-NH_2

Biological and Synthetic Porcine Secretin

H-His-Ser-Asp-Gly-Thr-Phe-Thr-Ser-Glu-
Leu-Ser-Arg-Leu-Arg-**Asp-Ser**-Ala-Arg-
Leu-Gln-Arg-Leu-Leu-Gln-Gly-Leu-Val-NH_2

Figure 1. Chemical structure of human and porcine secretin. The amino-acid sequence of human secretin is shown above, while that of biological and human porcine secretin is shown below. The two structures differ at amino-acids number 15 and 16 as shown.

equivalent to 1 CU of the biologically-derived product. These relationships were further confirmed in a study of exocrine pancreatic responses in normal healthy subjects.[13]

The current study was designed to assess the pharmacological effects, diagnostic efficacy and safety of the two synthetic products compared with the previously available biologically-derived product in patients with an established diagnosis of Zollinger–Ellison Syndrome. Secretin testing for tissue-confirmed Zollinger–Ellison Syndrome with the biologically-derived porcine product at a dose of 2 CU/kg is positive in 93%, 85–89% and 78–93% of all patients using the Federal Drug Administration (FDA)-approved criterion as described by Deveney, the National Institutes of Health criterion as described by McGuigan and Wolfe, and the Lamers and van Tongeren diagnostic criterion, respectively.[7, 9, 10, 14]

MATERIALS AND METHODS

The overall study design was a three-way, randomized, single-blind, active-controlled, three-treatment, Latin-squares crossover, IRB-approved, single-centre study in patients with a proven diagnosis of Zollinger–Ellison Syndrome. Written, informed consent was obtained from prospective patients who underwent a screening evaluation consisting of a complete medical history and physical examination, review of medications, and urine pregnancy testing (in female subjects of childbearing age) at least 7 days prior to administration of the study drug. Zollinger–Ellison Syndrome was confirmed in all patients if they had clinical and laboratory findings consistent with the diagnosis and a previously documented positive secretin stimulation test with biologically-derived secretin, or a positive tissue diagnosis of a neuroendocrine tumour.

Eligible patients were randomly assigned to a specified sequence for each of the three treatments in a single-blind fashion. A research pharmacist not involved in the clinical aspects of the study reconstituted the study drug and dispensed doses in a blinded syringe. Biologically-derived porcine secretin was reconstituted according to the directions in the package insert and administered at a dose of 2 CU/kg. Synthetic porcine and human secretins were reconstituted in 8 mL of sterile normal saline for injection and administered at the equivalent doses of 0.4 μg/kg. Each patient received an initial intravenous test dose of 0.1 mL of the specific study drug and was observed for 1 min to document the absence of an allergic reaction. Thereafter, the full test dose of drug was administered intravenously over 1 min. Blood draws for serum gastrin determination were obtained immediately before the secretin administration and then 1, 2, 5, 10, 15 and 30 min later. The primary end-point for a positive diagnosis of Zollinger–Ellison Syndrome by secretin testing was an increase in serum gastrin concentration by more than 110 pg/mL, as defined in the package insert for the biologically-derived product. Secondary end-points consisted of a 50% increase in serum gastrin concentration and a 200-pg/mL increase in serum gastrin concentration. The washout period between studies varied from hours to days, with the longest interval between testing of 62 days. One patient had all three tests done on the same day with a 2.5-h interval between administrations. Another patient had two tests on one day and the third test 24 h later. A third patient had her first two tests separated by 21 days and her third test 2.5 h later. The three remaining patients had all their studies performed on separate days.

Fasting serum gastrin determinations were performed by a single outside laboratory, Associated Regional and University Pathologists, Inc. (ARUP, Salt Lake City, Utah) contracted with the University of Pennsylvania. Each assay was run with a positive and negative control and all samples were diluted into the normal range permitting more accurate optical density measurements

corresponding with the linear portion of the assay curve. The gastrin serum assay is a double-antibody radioimmunoassay. The commercial kit is manufactured by Diagnostic Products Corporation (Los Angeles, CA). The assay has excellent reproducibility throughout the range of clinical interest, with intra- and interassay CVs of 5% and 7% at the upper reference limit. The detection limit is approximately 4.5 pg/mL (4.3 μIU/mL).

The small number of study subjects permitted only descriptive comparisons of diagnostic accuracy for each of the three secretin formulations. However, a parametric analysis of variance using the Proc GLM procedure from SAS along with the LSD multiple comparison procedure was employed to compare serum gastrin results for each of the three active secretin products. Baseline serum gastrin comparisons were performed using a model that included the factors of product and patient. For the post-baseline time points, the change from baseline was analysed using a repeated measure analysis of variance with product, patient, and time point as the factors. The mixed procedure in SAS was used to analyse these data with significance based on a paired *t*-test.

RESULTS

Table 1 lists the demographic and clinical characteristics of the six patients enrolled in the study. The mean age was 50 years, two were male, four had multiple endocrine neoplasia type I, and the mean fasting serum gastrin concentration was 4419 pg/mL, with a range from 113 pg/mL to 22200 pg/mL. A firm tissue diagnosis of Zollinger–Ellison Syndrome was available in five patients and one patient had widely metastatic disease. Basal acid output measurements were available for all patients with a mean value of 24.4 mEq/h and a range from 11.1 mEq/h to 45 mEq/h. Maximal acid output measurements were only available in two patients (38 mEq/h and 81 mEq/h). Two patients had previously undergone secretin testing with the biologically-derived product (both were positive).

Figure 2 illustrates the results of secretin testing for all six patients with each secretin formulation. Using the FDA-approved criterion for a positive diagnosis with biologically-derived porcine secretin, all six patients had positive tests with all three formulations (Figure 2, left column). Using the more stringent NIH criterion, positive results persisted in five out of six patients using biologically-derived secretin, in all six patients using synthetic porcine secretin, and in four patients using synthetically derived human secretin (Figure 2, middle column). Using a 50% increase in serum gastrin to designate a positive test, all six patients had positive tests with biologically derived secretin, all six had positive tests with synthetic porcine secretin, and four had positive tests with synthetic human secretin (Figure 2, right column).

Figure 3 illustrates the number of positive tests using the three predefined criteria for a positive result. There was complete agreement between all three tests using a 110-pg/mL increase in fasting serum gastrin to define positivity. For a 200-pg/mL increase, the agreement was 67%, with the synthetic human secretin negative when the other two tests were positive on one occasion and with the synthetic porcine secretin positive when the other two tests were positive on another occasion. For the 50%

Table 1. Clinical characteristics of the six Zollinger–Ellison Syndrome patients in the study

Patient no	Age (years)	Gender (M/F)	Disease duration* (years)	MEN-1 (Y/N)	FSG† (pg/mL)	Tissue diagnosis (Y/N)	Prior secretin test§ (pg/mL)	BAO (mEq/h)	MAO (mEq/h)
1	72	F	3	N	343 ± 49	N	ND	18.7	81
2	48	F	18	Y	113 ± 36	Y	209	11.1	38
3	44	M	16	Y	2860 ± 450	ND	21	ND	
4	46	M	1	N	122 ± 46	Y	ND	37.3	ND
5	28	F	1	Y	876 ± 311	Y	1007	45	ND
6	63	F	13	Y	22200 ± 2584	Y	ND	13.5‡	ND

MEN-1, Multiple endocrine neoplasia syndrome type 1; FSG, Fasting serum gastrin; BAO, Basal acid output; MAO, Maximal acid output; ND, Not done.

*Defined from onset of initial symptoms.

†Mean and standard deviation of the three baseline measurements before each secretin test.

‡This measurement was made in the presence of antisecretory therapy (ranitidine 1 mg.h/kg).

§The absolute increase in serum gastrin level is given.

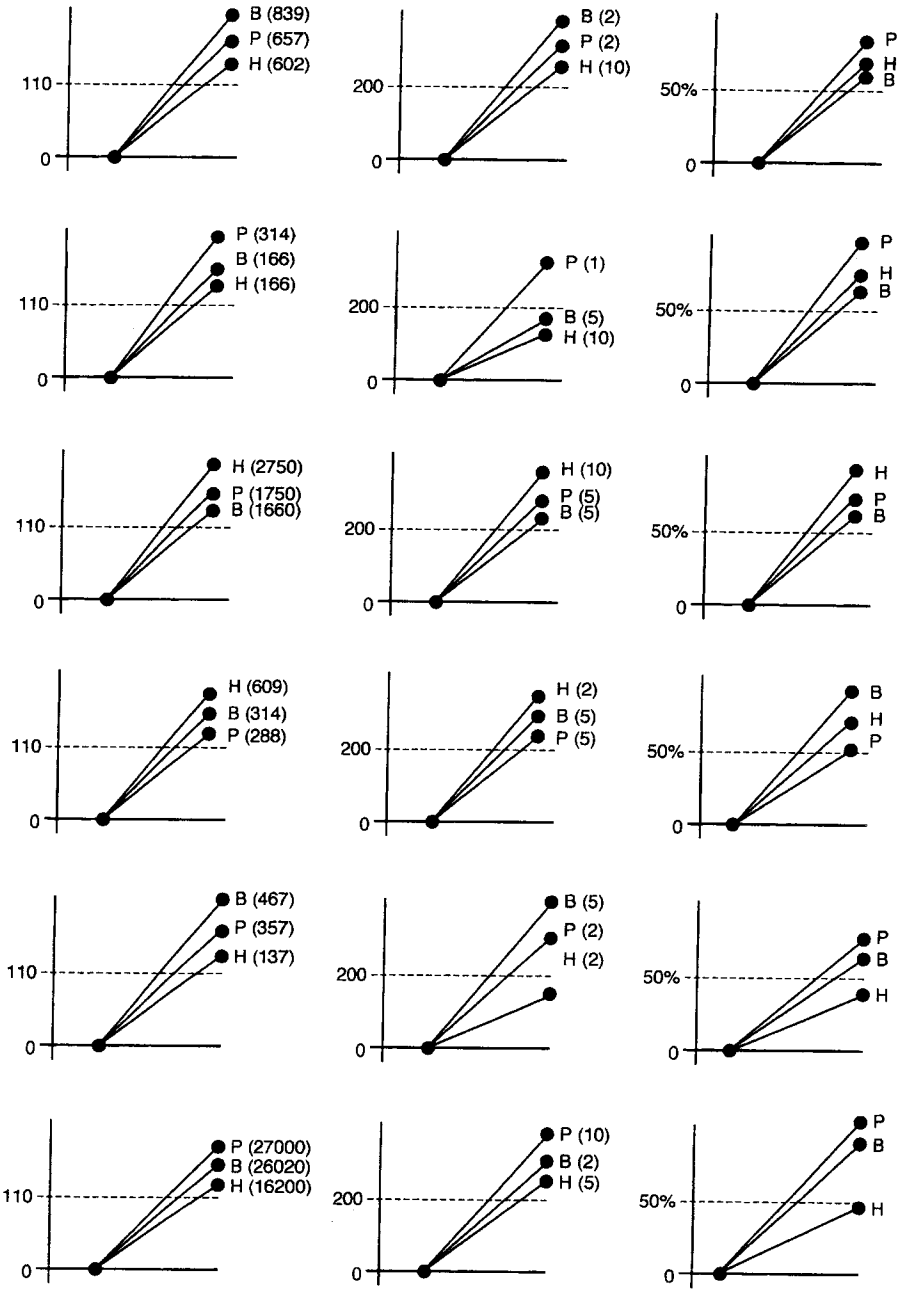

Figure 2. Individual secretin test results in the six patients enrolled in the current study. In each panel the y-axis reflects the change in serum gastrin concentration from baseline (left portion of x-axis) to maximum (right portion of x-axis). The left column of panels shows results using a serum gastrin increase of 110 pg/mL to reflect a positive result.[9] The middle column shows results using a serum gastrin increase of 200 pg/mL to reflect a positive result.[13] The right column shows results using a serum gastrin increase of 50% to reflect a positive result.[10] In each panel, the horizontal dotted line corresponds with the cut-off for a positive test. The numbers in parentheses next to the maximum attained correspond with the maximal increase in serum gastrin with each test article (left column) and the time point (in minutes after secretin injection) at which this occurred (middle column). B, Biologically derived secretin; P, Synthetic porcine secretin; H, Synthetic human secretin.

increase criterion, the overall agreement was also 67%. The biologically-derived porcine secretin and the synthetic porcine secretin were in complete agreement, while synthetic human secretin was negative on two occasions when the other two tests were positive.

Using all forms of secretin for administration, the time to peak serum gastrin concentration after secretin injection always occurred within 15 min; it occurred in 17 out of 18 (94%) studies by 10 min, and in 14 out of 18 (77%) studies by 5 min. Analysis of variance showed an extremely close correlation for serum gastrin levels at all times following secretin injection for all three treatments. Table 2 shows changes in serum gastrin concentration (as a percentage of the baseline value) at various time-points after secretin injection with each of the three study drugs. Three-way comparisons of serum gastrin levels with biologically derived porcine secretin, synthetic porcine secretin, and synthetic human secretin showed a single statistically significant difference in serum gastrin concentration. This was in the comparison between

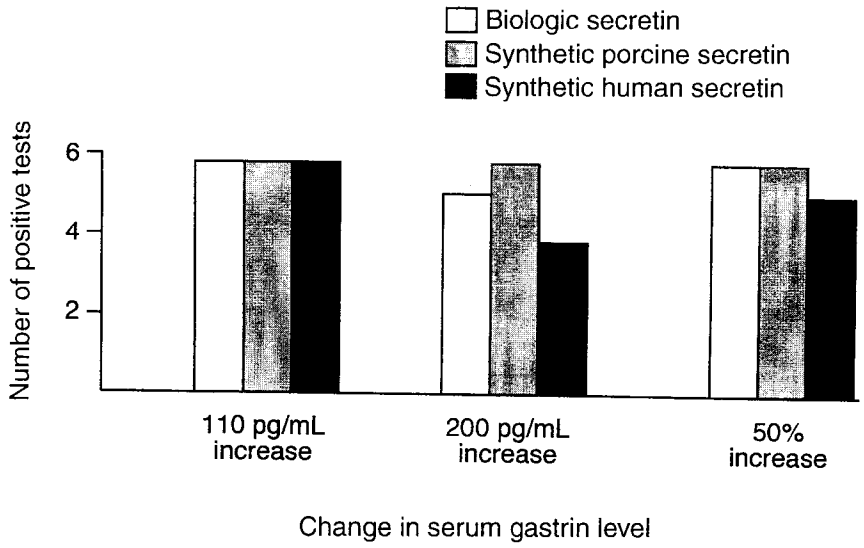

Figure 3. The number of positive secretin tests with each of the three secretin products tested according to the FDA criterion (left columns);[9] NIH criterion (middle columns);[13] and Lamers and Van Tongeren criterion (right columns).[10]

synthetic human secretin and synthetic porcine secretin in terms of change from baseline at 15 min ($P = 0.0274$; Table 2). Statistically significant changes from baseline occurred at 1, 2 and 5 min for biologically-derived porcine secretin and at 2 and 5 min for both synthetic porcine secretin and synthetic human secretin, in keeping with the expected time curve for positive secretin testing. All three agents were well-tolerated by all patients without any adverse events. There were no allergic responses noted.

DISCUSSION

Synthetic porcine secretin and synthetic human secretin are both excellent diagnostic agents for secretin testing in patients with Zollinger–Ellison Syndrome. The results of this study demonstrate an efficacy (sensitivity) that is comparable to biologically-derived porcine secretin which is no longer available in the United States. Although the current study had only six patients, the results suggest that either synthetic substance can be used for diagnosis instead. This study was not designed to assess the specificity of secretin testing for Zollinger–Ellison Syndrome. For such an analysis it will be necessary to test known non-Zollinger–Ellison Syndrome patients to determine the rate of false positivity. This important analysis will need to be carried out in time. However, we feel that the specificity of synthetic secretin testing for Zollinger–Ellison Syndrome will also be high because we employed equipotent doses of synthetic secretin in comparison with biologically-derived secretin according to the validated cat bioassay model. This relationships was confirmed in a study of exocrine pancreatic responses in normal healthy subjects.[13] We can think of no reasons

Table 2. Per cent increase from baseline serum gastrin level after secretin administration

Drug	Sample time (minutes)	Mean (%)	Standard deviation (%)	*P*-value
sHs	1	176.8	68.4	N.S.
sPs	1	229.3	199.0	N.S.
bPs	1	158.0	18.7	N.S.
sHs	2	226.3	133.3	N.S.
sPs	2	254.3	132.7	N.S.
bPs	2	255.4	92.6	N.S.
sHs	5	221.3	132.9	N.S.
sPs	5	247.0	87.1	N.S.
bPs	5	247.7	132.8	N.S.
sHs	10	218.8	101.0	N.S.
sPs	10	216.2	69.7	N.S.
bPs	10	202.3	113.2	N.S.
sHs	15	152.6	84.6	0.0274
sPs	15	255.0	89.4	0.0274
bPs	15	197.4	72.3	0.0274
sHs	20	165.0	61.8	N.S.
sPs	20	172.3	43.0	N.S.
bPs	20	166.5	68.0	N.S.
sHs	30	128.0	42.1	N.S.
sPs	30	131.0	51.8	N.S.
bPs	30	130.0	35.2	N.S.

sHs, synthetic human secretin; sPs, synthetic porcine secretin; bPs, biologically derived porcine secretin; N.S., not significant.

why secretin receptors on antral G-cells may behave any differently from similar receptors on pancreatic acinar cells in this regard.

Secretin testing is not an essential component for the diagnosis of Zollinger–Ellison Syndrome. In fact, the recent availability of functional imaging with Octreo-Scanning coupled with the prior lack of general availability of secretin, may dampen the enthusiasm for this test.[15] Despite this, however, there is a clear indication for the need for secretin testing in certain patients with suspected Zollinger–Ellison Syndrome, especially if baseline serum gastrin levels are only modestly elevated.[1–3, 7] Furthermore, because the sensitivity and specificity of secretin testing is at least 90%, the most cost-effective approach may be to only perform OctreoScanning after a positive secretin test result has been obtained.[7–11] The results of the current study are strongly in favour of either of the two synthetic substances being useful.

To put the above conclusion in correct perspective, a few points must made. First, this was a small study, and it is conceivable that clinically significant differences between the various products may become apparent as more patients are tested. The larger study comparing secretin with calcium infusion testing in the United States included 80 patients.[7] However, there are no other studies available in Zollinger–Ellison Syndrome patients specifically comparing the various forms of secretin.

Second, in the current study we decided not to discontinue the antisecretory therapy in any of our patients because we did not want to run the risk of losing control of their acid output and potentially having complications associated with this. False-positive secretin testing has been described in the presence of achlorhydria and it is conceivable that some of the results in these patients may be positive on the basis of achlorhydria (because they were all still taking oral medications) and not due to the gastrinoma *per se*.[16] However, we feel it is unlikely that these patients were all achlorhydric, and their acid output measurements obtained previously (data not shown) generally showed some acid output on drug. The precise cause for false-positive secretin tests in achlorhydric patients is unclear. Achlorhydria itself is a potent stimulus for gastrin release.[1–3] Therefore, it would be surprising to expect that secretin injection in this situation would lead to a further significant increase in the serum gastrin concentration. However, one of the limitations regarding the use of radioimmunoassays for serum hormone assessment is that optical density measurements are only accurate within a narrow range. It is thus essential to dilute elevated serum specimens into the normal range, permitting more accurate optical density measurements corresponding with the linear portion of the assay curve. Small volumetric errors made with serial dilutions in patients with massively elevated serum gastrin levels from achlorhydria may lead to large changes in serum gastrin measurements. This may explain the phenomenon of false-positive secretin testing under achlorhydric conditions.

A third concern about this study may be that there was insufficient washout between drug administrations. The serum half-life of secretin is less than 5 min, and waiting 2.5 h at a minimum between administrations, we feel, is unlikely to have affected results, because this is over 30 half-lives.[17] Furthermore, analysis of the secretin test results showed that all serum gastrin levels were not significantly different from baseline values by 30 min after secretin administration (Table 2).

In conclusion, either of the two synthetic secretin products evaluated in the current study appear to be useful in confirming the diagnosis of Zollinger–Ellison Syndrome and will likely fill the void left by removal of the previously used biologically-derived product from the United States market.

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