INCRETIN THERAPY AND ISLET PATHOLOGY – A TIME FOR CAUTION

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The past twenty-five years has seen the introduction of a number of new classes of medications for treating type 2 diabetes. The primary goal of these drugs is to safely lower plasma glucose in order to simultaneously reduce vascular complications and improve quality of life.

It is equally critical to demonstrate an agent's therapeutic effectiveness as it is to prove its safety. Frequently, this determination of safety occurs in the very early stages of drug development. However, in some instances, it is only after sufficient clinical experience has been obtained that the potential for harm becomes apparent. Recent examples include two therapeutically effective thiazolidinediones: troglitazone, which increased the risk of hepatic injury (1), and rosiglitazone, which a meta-analysis suggested increased the risk of cardiovascular events (2).

Two recently introduced classes of glucose-lowering agents increase incretin action. One is the glucagon-like peptide-1 (GLP-1) receptor agonists. In 2005, exenatide was the first of these agents to be introduced (3). The other is the dipeptidyl peptidase-4 (DPP-4) inhibitors, of which sitagliptin was initially approved in 2006 (3). These medications are widely used, and are associated with a reduced risk of hypoglycemia and weight gain. This, despite the fact that they may increase the risk of pancreatitis (4; 5), a condition that is known to be more common in type 2 diabetes (6).

In this issue of *Diabetes*, authors from Florida and California together report pancreatic morphology from a limited series of samples from diabetic individuals who did or did not receive incretin therapy and a cohort not known to have diabetes (7). Seven of the diabetic samples were from patients who received sitagliptin and one exenatide, all for unknown periods

of time beyond one year. Pancreas was procured from brain dead organ donors by the Network for Pancreatic Organ Donors with Diabetes (nPOD), a collaborative resource funded by the Juvenile Diabetes Research Foundation to promote type 1 diabetes research (http://www.jdrfnpod.org).

Aside from confirming that diabetic patients who had not received incretin therapy had reduced numbers of ß-cells, the study offers some new observations. First, the mass of endocrine cells was greater in subjects receiving incretin therapy. ß-cell mass was increased six-fold in diabetic subjects receiving an incretin-based medication versus those who were not, and was three-fold greater than in non-diabetic donors. Further, α-cell mass was increased five-fold in those with diabetes treated to enhance incretin action compared to those who were not, with mass in the non-treated diabetic group being comparable to those without diabetes. The increase in mass of these two endocrine cell types was due primarily to an increase in cell number, as cell size was similar across groups. However, the number of ß- and α-cells undergoing replication did not differ among the three groups. Second, insulin-immunoreactive cells related to ducts were present in all three groups, and did not differ in frequency in the two diabetic groups. Glucagon-immunoreactive cells were present in ducts or the periductal region and formed intraductal luminal projections as observed in chronic pancreatitis. A proportion of insulin positive cells were also positive for glucagon, the proportion being increased in both diabetic groups, but higher in those that received sitagliptin or exenatide. Third, pancreatic mass was increased by about 40% in diabetic patients receiving incretin-based therapy. This increase in mass was accompanied by an increase in proliferation of exocrine cells as well as dysplastic changes in the form of intraepithelial neoplasia, the latter increased a little over two-fold in those with diabetes

who received incretin therapy versus those who did not and was frequently associated with ductal α-cell complexes. Finally, three subjects treated with sitagliptin had glucagon producing microadenomas and one of them also had a glucagon producing neuroendocrine tumor. Based on these observations, the authors suggest it is time for caution and that additional work is required to better understand these changes and perhaps learn how to harness some of them for therapeutic benefit.

Aspects of the current study and the work of others need to be considered in parallel. First, substantial differences exist between the two diabetic groups. Subjects in the control group that did not receive incretin-based therapy were 18 years younger, 67% were female, five were diagnosed with diabetes at the age of 20 or younger, and two died of diabetic ketoacidosis. Further, five were not receiving glucose-lowering medications with the other seven on a single agent, which for four was insulin. Among those who received incretin-based therapy, only 25% were female, the group had a longer duration of diabetes, and seven of the eight subjects were using two or more medications to treat their diabetes. Thus, is the increase in pancreas mass in those receiving sitagliptin or exenatide due to these medications or is the increase more a function of some of those not receiving the medications actually having type 1 diabetes, a suggestion consistent with the greater use of insulin and much younger age at diagnosis in the latter group? This is important as magnetic resonance imaging studies have shown that pancreatic volume in subjects with type 1 diabetes is reduced by 26% within months of diagnosis and by 48% after at least 10 years of the disease (8), an observation supported from weights of pancreas samples in the nPOD resource (9). Further, could the differences in body size, sex, and age be confounders that explain some of the differing morphometric observations in the three

groups? A second consideration involves the observation that the profound increase in ß-cell mass in those exposed to incretin-based therapies was not due to an increase in ß-cell replication. Given that the small difference in ß-cell size cannot account for this change, it is not clear how this ß-cell mass increase is occurring. Could it be due to the therapy reducing ß-cell apoptosis (10; 11), effectively negating the increased apoptosis observed in type 2 diabetes (12; 13)? Such information would be valuable and would help us better understand the current findings. Or, is the difference in ß-cell, and possibly α-cell mass, again related to an imbalance in the types of diabetes in the two groups of diabetic subjects? Alternatively, could it be related to the preterminal clinical status of the donors, a factor that has been demonstrated to increase the rates of replication of both endocrine and non-endocrine cells in a series of 363 human organ donors on prolonged life support (14)? Third, the morphological abnormalities based on glucagon staining included microadenomas in 37.5% of the diabetic subjects treated with an incretin-based therapy and a glucagon producing neuroendocrine tumor in one of the seven who received sitagliptin. In contrast, the prevalence of pancreatic endocrine tumors is extremely low, being estimated at 0.0005% (15). Even assuming a lower prevalence of glucagon microadenomas–say 10%– and the millions of patient years of exposure to these agents, by now, would one not have expected reports of an increase in glucagon-related abnormalities in pancreas samples obtained at biopsy or autopsy and/or symptoms of glucagon excess beside hyperglycemia in diabetic patients on these classes of medications? Fourth, in subjects who have undergone gastric bypass surgery post-prandial GLP-1 levels are increased more than three-fold (16), levels in the range of, or greater than those observed with DPP-4 inhibition (17). Further, the hyperinsulinism observed in post gastric bypass subjects results from an increase in GLP-1-stimulated insulin secretion (18), with those experiencing hypoglycemia not having evidence of either increased ß-cell mass or

formation as long as eight years after surgery (19). As many of these post-surgical subjects will have had diabetes and long-term exposure to increased levels of endogenous GLP-1, could one have expected somewhat similar findings in both endocrine and exocrine tissue in these patients to the diabetic patients in the current study? Or, are the findings confounded by changes in body mass and may be an observation yet to come? Could it be–as the authors suggest– that differences in the local production of GLP-1 may exist, for which some immunostaining could have provided useful support? Fifth, while human data are always more valuable than findings in animals, we should not simply ignore substantial preclinical work that fails to substantiate a link between pancreatitis, undesired islet cell proliferation and incretin-based therapies (20; 21). This includes a consistent absence of change in the morphology and mass of α-cells in animals treated with incretin-based therapies (11; 22), in contrast to marked α-cell hyperplasia and hyperglucagonemia in the mice with glucagon receptor ablation cited by the authors (23; 24). Further, it is difficult to find literature substantiating the contention that partial reduction of glucagon secretion leads to compensatory α-cell hyperplasia.

The Food and Drug Administration's (FDA) requirement that evidence of cardiovascular safety be provided for new glucose-lowering agents means that all medications developed to enhance incretin action are or will be evaluated in long-term clinical trials (Table). In addition, a long-term, NIH-funded study will compare two of them (sitagliptin and liraglutide) to the sulfonylurea glimepiride and insulin glargine as add-on therapy to metformin in patients with type 2 diabetes (25). A valuable byproduct of these studies will be the opportunity for adjudicating clinical events related to pancreatic pathology, be it pancreatitis or pancreatic malignancy. This approach will surely be the most informative yet, providing data obtained in a rigorous manner in

patients with thousands of person-years of exposure. As none of the independent data and safety monitoring boards overseeing the ongoing studies has terminated any of them prematurely for cause, it is doubtful they are currently observing a worrisome signal of excess pancreatic malignancy. Should there be insufficient events in each individual study, pooled data could be used for meta-analyses of these critical outcomes.

As the type 2 diabetes epidemic continues worldwide, it would seem prudent to be cautious given the findings of the current study. The morphological findings reported in this study should prompt the FDA and independent investigators to undertake thorough examinations of these and other pancreatic samples from patients with type 2 diabetes carefully matched for age, sex, duration of disease and concomitant therapies who have and have not been exposed to incretin-based therapies. Further, they should re-analyze currently available data from all clinical trials with these agents. Sound clinical decision making requires use of reproducible scientific data from well controlled rigorous experiments, carried out with carefully matched control groups. In this regard, the current single morphological study in a small number of poorly matched subjects is sufficient to raise important questions and prompt additional investigation. However, the current level of evidence falls short of that required to prematurely banish two novel therapeutic classes that have thus far proven to be valuable in treating type 2 diabetes.

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Table 1: Long-term studies examining the safety of incretin-based therapies

Medication	Study Name	ClinicalTrials.gov Identifier	Comparator	Estimated Number of Subjects	Study Start Date	Estimated Study End Date
DPP-4 Inhibitors						
Alogliptin	EXAMINE	NCT00968708	Placebo	5,400	October 2009	December 2013
Linagliptin	CAROLINA	NCT01243424	Glimepiride	6,000	October 2010	September 2018
Saxagliptin	SAVOR-TIMI53	NCT01107886	Placebo	16,500	May 2010	July 2013
Sitagliptin	TECOS	NCT00790205	Placebo	14,000	December 2008	December 2014
GLP-1 Receptor Agonists						
Duraglutide	REWIND	NCT01394952	Placebo	9,622	July 2011	April 2019
Exenatide	EXSCEL	NCT01144338	Placebo	9,500	June 2010	March 2017
Liraglutide	LEADER	NCT01179048	Placebo	9,340	August 2010	January 2016
Lixisenatide	ELIXA	NCT01147250	Placebo	6,000	June 2010	May 2014

Source: ClinicalTrials.gov accessed on 27 March 2013