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COMMENTARY

Incretin Therapy and Pancreatic Pathologies: Background Pathology Versus Drug-Induced Pathology in Rats

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It is essential that postmarketing surveillance be conducted for any new drug, especially if the drug is a trailblazer for a new class of compounds, so that possible safety concerns not detected during clinical trials can be brought to light. It is also usual for a new drug class to be first marketed as a "game changer," followed by its vilification for potential safety issues (usually accompanied by lawsuits). Over time, its true benefits and safety profile are refined, confirmed, or denied after it has been used in the general population by large numbers of patients with a wide variety of medical conditions. The whole process can take years and sometimes involves a seesaw of both easing and adding restrictions to its clinical use as witnessed in the recent easing by the U.S. Food and Drug Administration (FDA) of the restrictions on rosiglitazone (1). Even when a drug has been in use for a long time, its complete mechanisms of action may remain enigmatic; e.g., the basic mechanisms of action for biguanides are still being determined even though their glucose-lowering properties have been known for 85 years.

Two novel classes of compounds for lowering blood glucose in type 2 diabetes came on the U.S. market recently—glucagon-like peptide 1 (GLP-1) receptor agonists (exenatide in 2005) and dipeptidyl peptidase-4 (DPP4) inhibitors (sitagliptin in 2006). Since then, the FDA has approved other agents in these two drug classes: liraglutide, a GLP-1 receptor agonist, and vildagliptin, saxagliptin, linagliptin, and alogliptin, all DPP4 inhibitors. Though both drug classes are commonly referred to as GLP-1–based or incretin-based therapies and epidemiological studies often lump the two classes of drugs together in analyses, it needs pointing out that "incretin therapy" is not precise terminology. While exenatide and liraglutide are ligands exclusively of the GLP-1 receptor, the effects of DPP4 inhibition are not exclusive to GLP-1/GLP-1 receptor signaling pathways. DPP4 exists both as soluble and membrane-bound forms, including being bound on a subset of T lymphocytes where it becomes upregulated upon activation, and it cleaves x-proline and alanine dipeptides from the N-terminus of polypeptides. It has a diverse range of substrates including neuropeptides, growth factors, chemokines, and vasoactive peptides, in addition to glucose-dependent insulinotropic peptide (the first incretin to be isolated), GLP-1, and GLP-2 (2), as well as containing several protein-binding sites.

The controversies surrounding the possible association between incretin therapy and pancreatitis first surfaced with postmarketing reports of acute pancreatitis with exenatide use in 2007 and with sitagliptin use in 2009. These two drug classes are now subject to scrutiny for possible association with pancreatic pathologies, namely pancreatitis and pancreatic cancer, based on some recent publications on pathologies seen in humans and rats that had received drugs from these two drug classes as well as data from epidemiologic studies. The issues are complicated by the fact that these pathologies are already more common in the age-group taking the drugs as well as in type 2 diabetic patients.

One study examined the FDA Adverse Event Reporting System (FAERS) database and found a sixfold increase in reported pancreatitis as well as a significant increase in reported pancreatic cancer in patients taking either sitagliptin or exenatide compared with other diabetes therapies (3). However, the FAERS is used for reporting adverse events and is subjected to

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disproportionate reporting of specific events, especially those linked to publicity, and the FDA states that "FAERS cannot be used to calculate the incidence of an adverse event in the U.S. population" (4). Six epidemiological studies using large U.S. health insurance databases have examined the odds of admission to the hospital for pancreatitis if taking either of the two new classes of drugs (5–10). One out of the six found an association (10). That study used a large insurance database of more than 1 million people, and reported increased odds of hospitalizations for acute pancreatitis in patients with type 2 diabetes 64 years of age or younger that were prescribed either exenatide or saxagliptin. Results were not separated out for the individual drugs, but were grouped together as "GLP-1–based therapy" to achieve statistical significance. Compared with nonusers of exenatide and saxagliptin admitted with pancreatitis, saxagliptin and exenatide users that were admitted to the hospital for pancreatitis were heavier, significantly more of them smoked tobacco and drank alcohol, they had higher triglycerides, more of them had gallstones (114 vs. 17), and more of them were already diagnosed with various neoplasms (10).

In 2013, a group of researchers examined pancreata from three groups of individuals, 8 individuals with diabetes on incretin therapy (7 taking sitagliptin and 1 taking exenatide for a year and more), 12 with diabetes receiving other glucose-lowering medications including insulin, and 14 nondiabetic control subjects. Again, these two drugs are grouped together as "incretin therapy." The authors report expansion of the endocrine and exocrine compartments, and the latter was accompanied by increased proliferation and dysplasia, while the former showed α-cell hyperplasia (11). There are many limitations in this study (should an $n = 1$ for exenatide have ever been published?), including whether some of these individuals actually had type 1 diabetes. Furthermore, in rhesus monkeys, α-cell expansion occurs and there is increased production of glucagon and GLP-1 within islets in response to insulin resistance even before blood levels rise (12), and therefore the preexisting clinical condition of the patients, similar to the monkeys, may have been responsible for at least some of the pancreatic findings.

GLP-1 receptor agonists bind to GLP-1 receptors. No off-site targets have been proposed with these compounds. Although GLP-1 fragments may have nonclassical GLP-1 receptor–mediated effects, this is not an issue with the two agonists, exenatide or liraglutide, presently in human use. Activation of GLP-1 receptors leads to upregulation of the cAMP/cAMP-dependent protein kinase cascade, which is how agonists increase insulin secretion, restore first-phase insulin secretion, and replenish insulin in secretory vesicles (13). That GLP-1 receptor agonists also increase receptor-mediated, cAMP-dependent acinar secretion has been known since Raufman et al. (14) reported it in 1992. Therefore, it would not be surprising to see increased exocrine secretion with the compounds. However, that does not mean they cause pancreatitis and pancreatic cancer. In clinical medicine, we had a corollary for increased cAMP generation. McCune-Albright syndrome results from an activating mutation in the *GNAS1* gene encoding the α-subunit of the stimulatory G proteins (Gsα). The mutation results in endocrine cell hyperstimulation and cell proliferation because of autonomous, continuous increased levels of intracellular cAMP. But no gastrointestinal cancers have been reported in a very large cohort of patients referred to the National Institutes of Health that are the most severely affected with the disorder, and mutations in the gene have not been associated with pancreatitis (15). It would seem unlikely that discontinuous GLP-1 receptor activation by agonists should be more lethal than McCune-Albright syndrome. Additionally, we, as well as others, reported the large increases in circulating GLP-1 that occur after gastric bypass surgery, which could be as much as 10-fold greater than presurgery levels (16). At least 70,000 gastric bypass procedures were performed each year over the past decade (17), and yet there are no reports of excess pancreatic cancers/pancreatitis in this population. Though, again, α-cell expansion was seen in pancreata after bypass (16), this could have predated surgery.

DPP4 inhibitors have several targets besides the penultimate proline/alanine of incretins, many of which were known prior to DPP4 inhibitors becoming available as treatments. Even the mechanism underlying their glucose-lowering properties is not solid and it is an oversimplification to state that it is all due to boosting active GLP-1 levels in the circulation. One DPP4 substrate worth mentioning is chemokine stromal cell–derived factor 1 α/β (SDF-1). Uncleaved SDF-1 enhances recovery from hematopoiesis, is a factor in cell survival, increases efficiency of transplantation, and promotes tumor blood vessel growth (18,19). One can therefore propose that in a setting of preexisting neoplasia, DPP4 inhibitors may promote its growth through increased active SDF-1 (Fig. 1) in conjunction with other factors.

Because human pancreata from patients who received exenatide, liraglutide, and DPP4 inhibitors are scarce, nonhuman pancreata serve as surrogates to find out if pancreatic pathology really is an issue with these newer drugs. Results from animal studies have been mixed. Studies in rodents, Sprague-Dawley (SD) rats (20–23), human islet amyloid polypeptide transgenic (HIP) rats (22), and Pdx1-Cre;LSL-$Kras^{G12D}$ mice (23), with no more than 5–15 rodents in each incretin therapy treatment or control group, found association of incretin therapy with ductal hyperplasia, inflammation, and pancreatitis. In other animal studies using SD rats (over 400 in one study) (24,25), cynomolgus monkeys (24), Zucker diabetic fatty (ZDF) rats (25–27), Wistar rats (25), hZAPP transgenic mice (28), C57B1/6J mice (29), GLP-1 $receptor^{-/-}$ mice (29), and over 700 CD1 mice (24), no association was found between incretin therapy (GLP-1

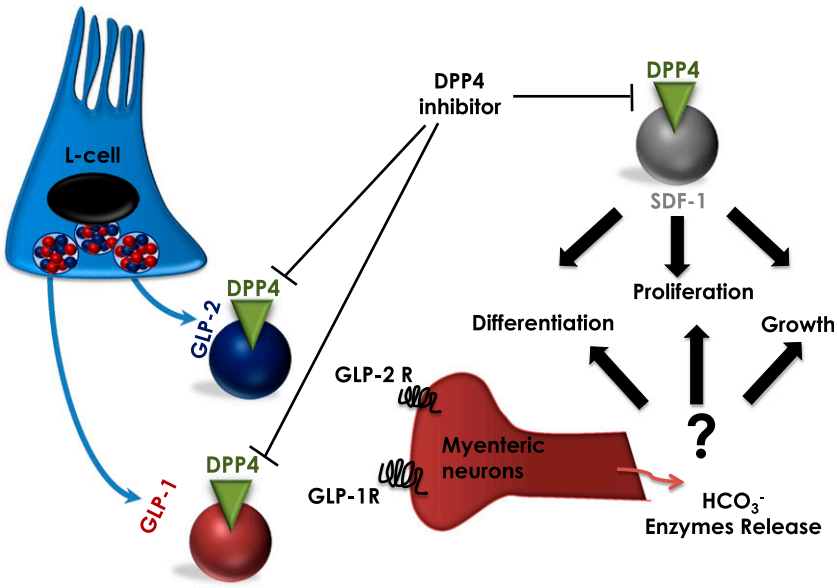

Figure 1—Schematic of DPP4 inhibition and its putative growth-promoting role in the exocrine pancreas. GLP-1 and GLP-2 are released from L cells in the gut in response to food. Their specific receptors are present on myenteric neurons. When engaged, the GLP-1 and GLP-2 receptors (R) activate myenteric neurons that, in turn, stimulate acinar and ductal secretions, as well as release of neurotrophic factors. DPP4 inhibition leads to increased amounts of active levels of GLP-1 and GLP-2 in the fasting state. Additionally, function of other growth factors, such as chemokine SDF-1, may be enhanced when DPP4 inhibition is in effect. Neuronal activation in the gut and enhanced levels of active SDF-1 could theoretically be additive and promote growth of preexisting neoplasia that is known to increase in frequency with aging and therefore in the human population likely to be prescribed DPP4 inhibitors.

receptor agonists or DPP4 inhibitors) and pancreatitis/pancreatic cancer.

Because of the high stakes involved for patients and companies, it behooves interested parties to sort out if some animals are already susceptible to pancreatic changes that might be confused with drug-induced pathology. Due to methodological questions raised concerning number of animals, selection of pancreatic sites, selection of subspecies, background pancreatic findings, and diabetic versus nondiabetic animals used in prior experiments, the work by Chadwick et al. (30) found in this issue attempted to address these questions by looking at the natural history of pancreatic findings in three strains of drug-naïve rats.

The authors evaluated the background pancreatic and biochemical findings of three male rat strains that had been previously used to investigate pancreatic side effects of the two classes of drugs: SD (commonly used in pharmaceutical toxicology studies and by academic researchers), ZDF (a diabetes-prone model due to a mutated leptin receptor), and HIP (a relatively new model of diabetes) rats. The rats, with access to food and water ad libitum, were given normal or high-fat diets (HFD) for 4 months: SD (n = 36 on normal diet and n = 36 on HFD), HIP (n = 36 on HFD), and ZDF (n = 36 on normal diet). The SD and HIP rats were about 12 weeks old and ZDF rats were about 8 weeks old at the start of the study.

The SD rats on HFD were significantly heavier (by 18%) than SD rats on normal diet at the end of the study. All the ZDF rats developed diabetes during the study, as expected, and half of them were killed prior to the end of study (between 9–15 weeks) because of medical complications associated with diabetes. Three of the HIP rats developed glucosuria with no significant increase in serum glucose. Amylase and lipase levels were higher at baseline in the ZDF rats relative to the other strains. Lipase levels increased modestly over time in SD and HIP strains, and amylase levels increased in all 3 strains.

None of the rats had macroscopic lesions of the pancreas. Pancreatic weights (adjusted to body weight) were lower in ZDF, SD HFD, and HIP HFD rats compared with SD normal diet rats. Pancreatitis was present in all strains (72% SD on normal diet, 42% SD HFD, 39% HIP, 6% ZDF) and in all parts of the pancreas (head, body, and tail). The lower level of pancreatitis in the ZDF rats was likely due to younger age. Peri-islet inflammation was present in both SD normal diet and SD HFD groups, a feature not described in pancreata from type 2 diabetic humans. Incidental pancreatic duct findings ranged from epithelial stratification/pseudostratification, epithelial papillary projections or cribriform epithelial pattern within the duct lumen, to pancreatic duct glands lined by columnar epithelium in about 20% of SD and ZDF rats on normal diets. No correlation between plasma glucose, fructosamine, HbA_{1c}, amylase, or lipase with incidence of microscopic exocrine pancreatic changes was found in any of the strains or any individual rat, illustrating that these rat models are prone to exocrine pancreatic incidentalomas independent of blood glucose levels.

The strengths of the study lie in the length of the study, the number of animals, and the care taken to

examine the whole pancreas. The biggest weaknesses are that rats of just one sex were used and water intake was not quantified. SD rats on so-called normal diets had more pancreatitis and heavier pancreata than SD rats on HFD. The normal diet was high in carbohydrate and protein and low in fat and most likely not normal for a rat, especially a bored, nonexercised male rat sitting on wire for 4 months in a cage with another bored male rat as his cage mate. One might imagine that the HFD leads to greater flow of acinar secretions and increased turnover of acinar contents; less viscosity of duct contents because of increased aqueous component from duct cells, resulting in less inspissation and blockage of flow of duct and acinar contents; and therefore lighter pancreata less prone to pancreatitis from duct obstruction than did the normal diet. Introduction of exenatide or liraglutide to a normal fed rat results in reduced food and fluid intake and could very well exacerbate the effects on the pancreas of a so-called "normal" diet. It is also possible that the SD HFD rats drank more water because the HFD is more palatable, and so animals were less dehydrated than normal fed animals.

Chadwick et al. (30) establish the natural history of biochemical and histological pancreatic changes that develop over time in three male rat strains on two different diets, sitting on wire, housed two to a cage. The findings underscore the importance of having the knowledge of the prevalence of background biochemical and histological pancreatic changes in the animal species and strains being studied for drug effects, under the conditions in which the animals are housed and fed. One possibility for the future is for the research community (i.e., academia, government, industries, other research institutions) to establish a common database, with prototype images and data, that would show the prevalence of these background biochemical and histological organ/tissue changes in different animal species and strains commonly used in drug studies.

"How now, a rat? Dead for a ducat, dead" (31). Hamlet mistakes Polonius for his uncle Claudius, whom he refers to as a rat and kills him. We must be careful not to kill a class of drugs because of a mistaken belief that background pancreatic findings in rats, which most likely are due to the feeding and housing conditions under which the animals are reared, are drug-induced.

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