Characterization of the Exocrine Pancreas in the Male Zucker Diabetic Fatty Rat Model of Type 2 Diabetes Mellitus Following 3 Months of Treatment With Sitagliptin

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Sitagliptin, a dipeptidyl peptidase-4 (DPP-4) inhibitor-based incretin therapy intended for the treatment of type 2 diabetes mellitus (T2DM), has not been linked to adverse effects on the pancreas in prospective clinical trials or in nonclinical toxicology studies. To further assess potential pancreatic effects, sitagliptin was studied in the male Zucker diabetic fatty (ZDF) rat model of T2DM. Following 3 months of oral dosing with vehicle, or sitagliptin at doses 3- to 19-fold above the clinically therapeutic plasma concentration, which increased active plasma glucagon-like peptide-1 levels up to approximately 3-fold, or following 3 months of oral dosing with metformin, a non-incretin-based reference T2DM treatment, the pancreas of male ZDF rats was evaluated using qualitative and quantitative histopathology techniques. In the quantitative evaluation, proliferative index was calculated in exocrine pancreatic ducts and ductules using computer-based image analysis on sections stained by immunohistochemistry for cytokeratin (a cytoplasmic epithelial cell marker) and Ki-67 (a nuclear marker of recent cell division). Relative to controls, sitagliptin treatment did not alter disease progression based on detailed clinical signs and clinical pathology assessments. Sitagliptin treatment did not result in pancreatitis or any adverse effect on the pancreas based on a qualitative histopathology evaluation. Proliferative index did not increase with sitagliptin treatment based on quantitative assessment of more than 5000 sections of pancreas, where control group means ranged from 0.698–0.845% and sitagliptin-treated group means ranged from 0.679–0.701% (\(P = .874\)). Metformin treatment was similarly evaluated and found not to have adverse effects on pancreas. (Endocrinology 155: 783–792, 2014)

The worldwide incidence of diabetes mellitus is increasing dramatically, especially in young people and in developing countries, to the extent that 1 of every 13 adults, or approximately 440 million individuals, are projected to have diabetes by 2030 (1, 2). During 2005–2006, two novel, incretin-based treatments for type 2 diabetes mellitus (T2DM) were introduced: glucagon-like peptide-1 (GLP-1) receptor agonists and dipeptidyl peptidase-4 (DPP-4) inhibitors (3). These treatments have subsequently gained wide therapeutic acceptance due to effective lowering of glycosylated hemoglobin (HbA1c), low hypoglycemia risk, no effect on body weight or promoting weight loss, and the capability of being combined with metformin (3, 4). Currently marketed GLP-1 receptor agonists are variants of the endogenously produced GLP-1 peptide that are resistant to otherwise rapid degradation by DPP-4. DPP-4 inhibitors prevent degradation of endogenously produced GLP-1 as well as the related peptide, glucose-dependent insulinotropic polypeptide (5). The increased persistence of endogenously produced GLP-1 and glucose-dependent insulinotropic polypeptide in circulation resulting from DPP-4 inhibition is differentiated from pharmacologic administration of a GLP-1 receptor agonist, with the latter associated with an increased
incidence of clinical gastrointestinal side effects, such as nausea (3, 4). In addition to demonstrated clinical efficacy in lowering fasting and postprandial glucose levels, incretin-based therapies may have a longer term antidiabetic effect. There is preliminary evidence in rodent models of T2DM and in vitro in cultured human pancreatic β-cells that suggests incretin-based therapies may slow the progressive loss of pancreatic β-cells, which is characteristic of the natural progression of T2DM (6–10).

Although there are proven benefits to incretin-based treatments of T2DM, questions have been raised regarding the possibility that long-term treatment might lead to pancreatitis and potential neoplasia (8, 11–16). Similar questions have been raised about a possible link to pancreatitis for other therapeutic classes of diabetes treatments in the past (17), possibly because diabetes constitutes an independent risk factor for pancreatitis and pancreatic cancer (18–21). Recent studies evaluating large databases of insurance claims demonstrated that incretin-based therapy poses no significant increase in risk for pancreatitis over that due to diabetes alone (18, 22, 23). Long-term studies of sitagliptin, a DPP-4 inhibitor, did not demonstrate a risk of pancreatitis or neoplasia when administered to rats up to 56-fold or to mice at up to 68-fold over the human therapeutic plasma concentration, to dogs in a chronic 9-month study, or to monkeys in a 3-month study up to 28-fold above the human therapeutic plasma concentration (17). A similar lack of evidence for pancreatic risk in animal studies has been reported for GLP-1 receptor agonists (24, 25). Two prospective studies using supratherapeutic doses of currently marketed GLP-1 receptor agonists (exenatide and liraglutide) in the Zucker diabetic fatty (ZDF) rat model of T2DM did not demonstrate a direct or indirect adverse effect on the exocrine pancreas in a well-characterized rodent model of T2DM. Specific end points included assessments for pancreatitis, pancreatic ductal metaplasia, and proliferative rate of pancreatic duct and ductular epithelial cells. The doses used provided plasma concentrations in excess of the clinical therapeutic plasma concentration (8.5 μM · hour) (29), inhibited DPP-4 activity, and increased active plasma GLP-1 levels.

### Materials and Methods

#### Animals

A histologic evaluation was performed on 300 male ZDF rats (Table 1). Six groups of 25 rats each received vehicle and were evaluated qualitatively by light microscopy and quantitatively by computer-based morphometric methods (database controls) to provide a robust historical control ZDF database. Six groups of 25 rats each were used in the pivotal evaluation of potential effects on the pancreas. In the pivotal study, 2 control groups received the same vehicle used to generate the database controls; 3 groups received sitagliptin as a phosphate salt monohydrate GLP-1 receptor agonists (exenatide and liraglutide) in the Zucker diabetic fatty (ZDF) rat model of T2DM did not demonstrate adverse pancreatic findings (26, 27). A pilot study of exenatide in our laboratory confirmed the published absence of an adverse pancreatic effect in the ZDF rat model (data on file).

The ZDF rat (fa/fa) model of T2DM is well characterized and widely used in diabetes research because it is known to share many of the common pathophysiologic hallmarks of T2DM in humans such as obesity, insulin resistance and glucose intolerance in liver and extrahepatic tissues, progressive hyperglycemia associated with loss of pancreatic β-cell mass, and impaired carbohydrate metabolism (28). In addition, the ZDF rat develops common comorbidities of T2DM found in humans, such as lenticular cataract, retinopathy, nephrosis, neuropathy, and impaired wound healing (28). The female Zucker rat does not consistently develop hyperglycemia (28) and therefore was not considered a useful model for this study.

The beneficial effects of sitagliptin on the endocrine pancreas in diabetic rodents have previously been demonstrated (8, 9). The present study evaluated whether or not sitagliptin produced a direct or indirect adverse effect on the exocrine pancreas in a well-characterized rodent model of T2DM. Specific end points included assessments for pancreatitis, pancreatic ductal metaplasia, and proliferative rate of pancreatic duct and ductular epithelial cells. The doses used provided plasma concentrations in excess of the clinical therapeutic plasma concentration (8.5 μM · hour) (29), inhibited DPP-4 activity, and increased active plasma GLP-1 levels.

### Table 1. Group Assignments for 300 Male ZDF Rats Evaluated Qualitatively by Light Microscopy and Quantitatively by Computer-Based Morphometric Methods

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of Rats</th>
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<td>Database controls</td>
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<td>Concurrent controls</td>
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<td>II</td>
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<tr>
<td>Sitagliptin (mg/kg/d)</td>
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<td>150</td>
<td>25</td>
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<tr>
<td>Metformin (mg/kg/d)</td>
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<td>450</td>
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Additionally, in separate arms of this study, 8 male ZDF rats were studied to determine the effect of 150 mg/kg/d of sitagliptin on total and active GLP-1 levels, and 36 male ZDF rats were studied to determine the effect of 150 mg/kg/d of sitagliptin on DPP-4 inhibition.
Charles River Laboratories. For groups intended for histopathology evaluation (including 2 concurrent vehicle control groups and 6 vehicle control groups evaluated to establish a ZDF-Leprfa/Crl male rat database), 25 rats were prospectively assigned to each group using a randomization protocol. Rats were pair housed in solid-bottom plastic box caging with contact bedding and provided PMI Rodent Diet 5008 (LabDiet), which is considered a high-fat diet, and tap water ad libitum. Animals were housed in environmentally controlled rooms with an approximately 12-hour light, 12-hour dark cycle. For the associated studies evaluating the pharmacologic effects of sitagliptin in ZDF rats, similar animal husbandry conditions were used, but the group size was 4 or 12 and the sitagliptin doses studied were 150 or 500 mg/kg/d corresponding to integrated plasma exposures (area under the curve [AUC]) of approximately 149 and 522 μM · hour.

Compound administration

The 300 male ZDF rats evaluated histologically were dosed once daily at 5 mL/kg body weight by oral gavage based on the most recent body weight. Control rats received vehicle as 0.5% (wt/vol) methylcellulose with 5 mM HCl in deionized water. Sitagliptin (chemically synthesized by the study sponsor, Merck & Co., Inc.) was administered as a suspension in 0.5% (wt/vol) methylcellulose with 5 mM HCl in deionized water. Doses of 30, 100, and 150 mg/kg/d of sitagliptin were chosen based on data from previous studies, suggesting that these doses would produce, respectively, 3, 10, and 17 times the human clinically efficacious exposure (AUC0–24 hours) of 8.5 μM · hour (29). Metformin (Framhispania) was administered as a suspension in 0.5% (wt/vol) methylcellulose in deionized water at 450 mg/kg/d based on the estimated maximum tolerated dose in male rats in the most recent marketing approval for metformin and targeting approximately 8-fold the human clinically efficacious exposure (30–32). Samples of sitagliptin and metformin dosing formulations were assayed for concentration and uniformity in week 1 and for concentration in week 12. All assay results were within the acceptable range (±15% of claim for a suspension) for concentration and uniformity. The same dosing procedures were used to evaluate the pharmacologic effects of sitagliptin in ZDF rats.

Clinical observations

For rats intended for histologic endpoints, body weight and food consumption data were collected once weekly. Physical sign observations were performed daily. Approximately 2 mL of blood was collected via the retro-orbital route under isoflurane anesthesia during weeks 4 and 12 for hematology and blood chemistry analysis. During week 12, an overnight urine collection was performed to measure volume, pH, and specific gravity. Prior to initiation of dosing and in weeks 2, 8, and 13, a nonfasted whole-blood sample was collected from the tail vein of each animal between 7:00 and 9:00 am before dosing to measure glucose level via a glucometer (LifeScan OneTouch Ultra Glucometer). In week 12, plasma (~350 μL per sample) was collected by tail vein for determination of circulating levels of sitagliptin or metformin at nominal times of 0.5, 1, 2, 4, 8, and 24 hours post dose from concurrent control and treated rats. Due to limitations of blood volume sampling from individual rats, samples for drug levels were collected using a scheme that used a subgroup of rats at each time point but resulted in an equal total volume collected from each rat over the time course. The assays for sitagliptin and metformin were conducted by a validated bioanalytical method (compliant with regulatory quality standards) (33) using liquid chromatography/tandem mass spectrometry. The 1-hour plasma samples were assayed from the 2 concurrent control groups and found not to have test article contamination.

Plasma DPP-4 inhibition (Merck & Co, Inc) and total and active plasma GLP-1 levels (Meso Scale Discovery) were measured in male ZDF rats intended for pharmacology endpoints in a group treated with sitagliptin at 150 mg/kg/d from samples collected at 0.5, 1, 2, 4, 8, and 24 hours post dose using a collection scheme similar to that described previously for DPP-4 inhibition, or at 0, 2, 4, 8, 12, 16, and 20 hours after a single dose for total and active GLP-1 levels.

Pathology evaluation

At study end, the rats were fasted overnight, weighed, anesthetized with isoflurane, and euthanized by caval exsanguination. A complete necropsy was performed, and pancreas and brain weights were recorded. For organ weight comparison, a trend analysis with multiplicity adjustment was used between sitagliptin-treated rats and concurrent controls. The entire pancreas from all rats was fixed at room temperature in 10% neutral buffered formalin for 36 hours ± 4 hours.

To ensure that each rat in a given treatment group made a balanced contribution to the total pancreatic area evaluated, 9 segments, representative of the entire pancreas from head to tail, were collected from all rats. Trimming was done using a systematic random sampling scheme to allow an unbiased and complete assessment of each pancreas. The 9 tissue segments to be evaluated from each rat were embedded in 3 paraffin blocks (3 segments per block), so that each block included a segment from the head, body, and tail of the pancreas.

An approximately 5-μm paraffin section of pancreas was prepared from the 3 blocks from each rat and stained with hematoxylin and eosin (H&E). A second approximately 5-μm adjacent section was prepared for immunohistochemical staining. In all cases, paraffin sectioning of blocks (microtomy) was performed the day before the immunohistochemistry staining process was initiated, so that the interval between microtomy and immunohistochemistry staining was similar for all slides.

The qualitative light microscopic evaluation conformed to Good Laboratory Practice Standard Operating Procedure methods, which are fully compliant with the best practices outlined by the Society of Toxicologic Pathology (34), and are the standard practice for generating nonclinical data submissions to support drug registration but do not involve blinded the pathologist to treatment group.

Cell proliferation evaluation

Proliferative index (PI) in pancreatic duct and ductular epithelial cells in the exocrine pancreas was determined for 300 male ZDF rats. To minimize lot-to-lot variability in reagents for concurrent control groups I and II and treated rats, supplies for immunohistochemical staining were purchased in bulk before study start. For all rats studied, slide deparaffinization and antigen retrieval were conducted in a single processing step in a module (Thermo Scientific Pretreatment Module) with precision temperature controls, and with sufficient capacity to hold all the
slides from one staining batch. A single immunohistochemistry stainer (DAKOCytomation Autostainer Plus) was used. To limit the impact of variability in immunohistochemical staining, all batch processing steps were conducted so that each dose group was equally represented in each processing batch. The immunohistochemical approach used was a dual-staining method using pancytokeratin (1:30,000; Abcam antibody 6401) and Ki-67 (1:1600; Abcam antibody 16667) antibodies. The pancytokeratin antibody preferentially stained the cytoplasm of epithelial cells in pancreatic ducts and ductules. The Ki-67 antibody stained the nuclei of recently mitotically active cells.

All 9 pancreatic sections stained immunohistochemically from each rat killed at study termination were digitally scanned with a digital whole-slide scanner (Aperio ScanScope XT) using a 20× objective and annotated (Aperio ImageScope). Analysis for PI was conducted on the entire section of pancreatic parenchyma from each of the 9 sections from each rat by the Cyto-nuclear Tool (Indica Laboratories) computer image analysis algorithm. Appropriate settings for the Cytonuclear Tool algorithm were determined in pilot experiments, and the settings were established prior to analysis. All PI measurements reported herein were made using identical algorithm settings using all the pancreatic tissue on the slides. Duct and ductular epithelial cells were identified by the presence of sufficient cytokeratin staining within a specified distance from the nucleus. PI was determined by dividing the number of recently mitotic epithelial cells (cells positive for pancytokeratin and positive for Ki-67) by the total number of epithelial duct and ductular cells (cells positive for pancytokeratin) and multiplying the resulting value by 100.

For rats evaluated at scheduled study termination, the 9 sections stained with H&E and the 9 adjacent sections stained immunohistochemically were evaluated qualitatively by a pathologist, and a peer review of all of these slides was conducted by a second pathologist. In the case of rats found dead during the study, pancreas sections were prepared and stained with H&E only, using the same methods as for the scheduled sacrifice rats. These rats were not included in the quantitative evaluation because pancreatic tissue from these animals was not suitable for immunohistochemical staining within the quality specifications required for a suitably accurate measurement of PI.

**Statistical analysis**

For hematology and serum biochemistry parameters, tests for normality (Wilk-Shapiro statistic) and homogeneity of variance (Levene’s test) were conducted on each parameter for each time interval. The Dunnett’s multiple comparisons test was conducted to determine statistically significant differences \( (P \leq 0.05) \) between individual treatment group and the concurrent control group I means. Mean active and total GLP-1 AUC were compared between control and treatment groups by Student’s \( t \) test \( (P \leq 0.05) \).

For the prespecified statistical plan, the significance of trends in pancreatic weight in sitagliptin-treated rats (ie, an increase or decrease with increasing dose of sitagliptin) was assessed by comparing absolute weight, percentage of body weight, and percentage of brain weight relative to control group I. P values were reported with adjustment (Dunnett’s) for multiplicity of tests. Statistical significance was set at \( P \leq 0.05 \).

For PI in the exocrine pancreas, one-way ANOVA model was fit to the angular transformation (arcsine square root) of PI, with the groups determined by the treatment regimen administered.

The null hypothesis that the PI does not increase with dose was tested against the alternative of an increasing dose trend using a linear contrast. This contrast effectively averages the 2 control groups.

**Results**

**Body weights, food consumption, hematology, and clinical chemistry**

There were no differences noted in body weight, food consumption, complete blood count (CBC), or urinalysis parameters in sitagliptin-treated rats. Fasting plasma glucose after 12 weeks (Supplemental Table 1 published on The Endocrine Society’s Journals Online web site at http://endo.endojournals.org) and nonfasting blood glucose (Figure 1) were consistent with diabetes mellitus and not significantly changed by treatment with sitagliptin. The slight decrease in fasting plasma glucose in week 4 in the group treated with sitagliptin 150 mg/kg/d vs the control group was not statistically significant \( (P > 0.05) \) (Supplemental Table 1). Anticipated age-dependent increases in fasting glucose in the ZDF model were observed.

Decreases in triglycerides observed in sitagliptin-treated groups weeks 4 and 12 were statistically significant \( (P \leq 0.05) \) at week 4 for sitagliptin 100 mg/kg/d (6.73 mmol/L [596 mg/dL]) relative to the control I (9.35 mmol/L [827 mg/dL]) and at week 12 for sitagliptin 150 mg/kg/d (8.17 mmol/L [723 mg/dL]) relative to the control I (10.17 mmol/L [900 mg/dL]). The general similarity between control and sitagliptin groups in body weight change, food consumption, and in blood chemistry measures suggests little difference in manifestation of T2DM in the ZDF rat model due to sitagliptin treatment. In-life study findings in 150 database control ZDF rats (Table 1) evaluated in preparation for the sitagliptin study were sim-
ilar to those of the 50 ZDF rats in concurrent control groups I and II.

**Sitagliptin plasma exposure kinetics and pharmacology**

The integrated sitagliptin plasma concentrations were $2.7 \times 10^3$, $12 \times 10^3$, and $19 \times 10^3$ the therapeutic clinical exposure ($\text{AUC}_{0–24}$) of $8.5 \mu\text{M} \cdot \text{hour}$ at doses of 30, 100, and 150 mg/kg/d, respectively, as summarized in supplemental Table 2. Sitagliptin pharmacokinetics in week 12 demonstrated adequate exposure margins relative to the therapeutic clinical plasma exposure.

In a separate group of male ZDF rats studied for pharmacology end points, treated for 12 weeks with sitagliptin $150 \text{mg/kg/d}$ (plasma $\text{AUC}_{0–24\text{hr}}$ of $149 \times 10^2 \mu\text{M} \cdot \text{hour}$ in week 12), DPP-4 inhibition was greater than 90% from 0.5 to 8 hours post dose, and greater than 80% at 24 hours post dose. Therefore, the integrated sitagliptin plasma concentrations achieved in the 150 mg/kg/d group in this study significantly exceeded the human therapeutic plasma concentrations and were sufficient to test the possibility that DPP-4 inhibition might affect the pancreas in this model.

After a single dose of sitagliptin at approximately 150 mg/kg/d ($n = 4$ group), levels of total GLP-1 were unchanged ($P = .590$), but levels of active GLP-1 were increased approximately 3-fold ($P = .003$). Plasma glucose levels were not significantly affected by treatment ($P = .439$) (Figure 2).

**In-life findings with metformin treatment and plasma exposure kinetics**

In the group treated with metformin at 450 mg/kg/d (plasma $\text{AUC}_{0–24\text{hr}}$ of $1430 \times 10^2 \mu\text{M} \cdot \text{hour}$ in week 12) there was an increase in mean body weight (+86 g) compared with controls (+44 g) over the course of the study, approximately 20% less weekly food consumption than controls, and approximately 40% greater increase in triglyceride levels compared with controls. At the end of the study, the fasting blood glucose in the metformin group was decreased (14.21 mmol/L [256 mg/dL]) compared with control group I (18.87 mmol/L [340 mg/dL]) ($P \leq .05$) consistent with less progression of disease in this group compared with other groups. The metformin $\text{AUC}_{0–24\text{hr}}$ of $1430 \times 10^2 \mu\text{M} \cdot \text{hour}$ provided an approximately 8-fold multiple of the $\text{AUC}_{0–24\text{hr}}$ at the maximum recommended clinically therapeutic dose of 2000 mg/d.

**Mortality, pancreas weight, gross changes, and histology findings**

Mortality was equally distributed across treatments (1 concurrent control, 1 sitagliptin-treated, 1 metformin-treated). In our laboratory many cases of spontaneous mortality in male ZDF rats are associated with apparent obstruction of the urinary tract characterized grossly by various combinations of dilation of the renal pelvis in kidney, distension of the ureters, dilation thickening and red discoloration of the bladder, swelling of the prepuce, and red discoloration of the penis. The 3 rats that died spontaneously in this study had combinations of such gross changes in the urinary tract. There were no sitagliptin-related changes in pancreas weight as absolute weight, a
percentage of body weight, or a percentage of brain weight (Supplemental Table 3). Based on gross examination and qualitative light microscopic evaluation, there were no treatment-(sitagliptin) related findings in the pancreas and no evidence of pancreatitis in any sitagliptin-treated rat. The incidence of exocrine pancreatic lobular atrophy and exocrine pancreatic acinar hyperplasia, 2 focal background pancreatic changes that exhibited a qualitative increase in Ki-67 staining, are summarized in Table 2. The incidence of lobular atrophy and acinar hyperplasia in groups of sitagliptin-treated rats was less than the highest incidence observed in control database groups (A–F) and concurrent control groups (I and II).

The incidence and severity of exocrine pancreatic lobular atrophy and acinar hyperplasia were comparable among control database groups, concurrent control groups, and treated groups (Table 2). These focal areas of change were included in the quantitative evaluation for calculation of PI.

In metformin-treated rats there was an approximately 20% increase in mean pancreas weight compared with concurrent control group I when evaluated based on absolute weight or when normalized to brain weight \( (P \leq .05) \), but no relevant difference when normalized to body weight \( (3.7\% \text{ increase}) \). Mean terminal body weight was increased in metformin-treated rats, and therefore the increase in pancreas weight on an absolute basis or normalized to brain weight was considered to be an adaptive response to the increase in body weight. There were no metformin-related gross or qualitative

| Table 2. Incidence of Focal Background Findings in Exocrine Pancreas (n = 25 per Group) |
|-----------------|-----------------|-----------------|
|                 | Control Database | Concurrent Control | Sitagliptin (mg/kg/d) |
|                 | A B C D E F      | I II             | 30 100 150            |
| Exocrine pancreas |                 |                 |                  |
| Lobular atrophy  | 0 1 1 3 2 3     | 2 1             | 2 0 2              |
| Acinar hyperplasia | 3 4 8 4 9 3   | 3 7             | 4 2 4              |

**Figure 3.** A representative example of pancreatic lobular atrophy, a common spontaneous degenerative finding in laboratory rats (H&E; scale bar in panel A, 100 \( \mu \text{m} \); scale bar in panel B, 50 \( \mu \text{m} \)) that exhibits qualitatively increased staining for Ki-67 in serial sections (IHC + hematoxylin; scale bar in panel C, 100 \( \mu \text{m} \); scale bar in panel D, 50 \( \mu \text{m} \)). Note that Ki-67 is most commonly expressed in small duct and ductule cells in foci of pancreatic lobular atrophy. IHC, immunohistochemistry.
light microscopic findings in the pancreas, and a similar incidence of lobular atrophy (2 per 25 rats) and acinar hyperplasia (6 per 25 rats) to control rats.

Quantitative histopathology evaluation of the exocrine pancreas

In all rats surviving to study end, PI was measured by means of a commercially available computer algorithm (Cytonuclear Tool, Indica Laboratories) applied to all of the pancreas tissue present on each slide and evaluated using algorithm settings established before the evaluation by optimization on slides from pilot studies. Use of predetermined algorithm settings and an automated data collection process encompassing the entire section avoided the introduction of observer bias into the data collection or the need to make estimates of PI based on subsamples. The algorithm identified nuclei based on size, shape, and intensity of hematoxylin staining (Figure 5, A and B). Ki-67–positive nuclei were identified by the presence of 3,3′-diaminobenzidine staining. Duct and ductular epithelial cells were identified by the presence of sufficient cytokeratin staining within a specified distance from the nucleus.

Visual inspection of the distribution of PI across the 6 control database groups, 2 concurrent control groups, and sitagliptin-treated groups (Figure 6, A and B; and Supplemental Table 4) confirms the uniformity of variability between groups as well as the similarity of means and medians. Mean and median PI for sitagliptin-treated groups were less than concurrent control group I. There was no increasing sitagliptin dose trend for PI (P = .874).

The mean ± SD PI for the metformin-treated group was 0.873 ± 0.295. Comparison between the metformin and control groups was not part of the prospective statistical plan, and meaningful comparison between the metformin group and other groups was complicated by metformin treatment-related differences in disease progression in this model, illustrated by differences in blood glucose changes, terminal body weight, food consumption, and pancreas weight (described in previous text).

Discussion

In the ZDF rat model of T2DM, sitagliptin did not adversely affect the pancreas across a range of integrated plasma concentrations that inhibited DPP-4 activity and exceeded the human therapeutic plasma concentration target by 3- to 19-fold. As anticipated, sitagliptin had no effect on total GLP-1 levels, but increased active GLP-1 levels approximately 3-fold. Both potential pharmacologic and toxicologic effects were evaluated, and there was no evidence of pancreatitis and no evidence of an increase
The assessments in this study were adequately controlled, complete, unbiased, and sufficiently powered (n = 25 per group at study start with 2 concurrent control groups and 6 control database groups) to detect small treatment-related effects. A range of sitagliptin doses were evaluated to explore the possibility of an inhibitory toxicologic effect of sitagliptin at high exposures on any of the measured endpoints and to increase the possibility of detecting a dose-dependent toxicity by a dose trend analysis. Although the anticipated pharmacologic effects of sitagliptin were observed in this study (DPP-4 inhibition and active GLP-1 increase), there was little effect of the observed pharmacology on progression of T2DM, likely due to the fact that diabetes in the ZDF rat is driven by insulin resistance (28). Consistent with this idea, metformin, an insulin sensitizer, did impact progression of T2DM in this study (discussed later in text). The observed lack of a notable effect of sitagliptin pharmacology on disease progression was a desired feature of the study design, because it allowed direct comparison between control and sitagliptin-treated groups with similar T2DM disease burdens. If sitagliptin treatment-induced pharmacology had notably altered progression of T2DM, then it would have been difficult to separate the beneficial secondary effects of sitagliptin pharmacology in the model from any observed toxicologic effects. Similar T2DM disease burdens between control and sitagliptin-treated groups allowed an evaluation for potential adverse sitagliptin effects in the face of hyperglycemia and T2DM comorbidities. The lack of adverse sitagliptin effects in rodents without a specific disease predilection has been documented elsewhere (17).

The ZDF rat model of T2DM that was used in this study has been widely studied by others, is well characterized, and shares many of the common pathophysiologic hallmarks of T2DM in humans. The absence of a detectable adverse effect with a DPP-4 inhibitor in this study is consistent with findings recently reported from nonclinical studies of GLP-1 agonists in ZDF rats (26, 27). Literature reports of a lack of adverse findings and no increase in PI in the pancreas in the ZDF rat model treated with exenatide were confirmed in a small pilot study (n = 12 per group) in our laboratory (unpublished results), in which up to 250 µg/kg/d of exenatide was administered for 3 months and mean fasting blood glucose in week 8 was 14.04 mmol/L (253 mg/dL) compared with a mean control value of 17.04 mmol/L (307 mg/dL). The lack of adverse changes in the pancreas in prospective studies in ZDF rats is consistent with results in nonclinical toxicology studies of DPP-4 inhibitors and GLP-1 agonists with nondiabetic animals (17, 24, 35) and is consistent with results from well-controlled epidemiologic studies of humans (18, 22, 23).

In the course of conducting the qualitative evaluation, 2 types of spontaneous focal background changes (exocrine pancreatic lobular atrophy and acinar hyperplasia) were identified in the exocrine pancreas with a qualitatively increased staining of nuclei for Ki-67 compared with unaffected exocrine parenchyma. Lobular atrophy, like that observed in this study, is recognized as a spontaneous change in laboratory rats used in nonclinical safety assessment studies and is commonly attributed to obstruction of a small duct draining the affected lobule (36). The increased staining of nuclei with Ki-67 in areas of lobular atrophy may reflect activation of a program of tissue repair in response to injury.

Similarly, focal acinar hyperplasia is a spontaneous change that is routinely observed in strains of laboratory rats typically used in nonclinical studies (36). However, the incidence of acinar hyperplasia is increased in aged rats, and an increased incidence is also reported in younger rats in association with feeding high-fat diets (37), such as the diet used in this experiment. Therefore, although the incidence of acinar hyperplasia in the high-fat diet ZDF rat model is higher than for some strains and feeding regimens used to conduct nonclinical safety assessment studies, the
increased incidence that was observed was anticipated (38). In the rat, acinar hyperplasia has been suggested to be part of a continuum of change that includes exocrine pancreatic neoplasia, which has been observed with increased incidence in rats fed high-fat diets or gavaged with corn oil vehicle in carcinogenicity studies (37, 39). Therefore, given the well-established links between the incidence of neoplasia in the laboratory rat and type of diet and food consumption (38), it is necessary to control for diet, food consumption, and body weight in studies with a prospective intent to assess cancer risk.

Because acinar hyperplasia in the rat has been suggested to be part of a continuum of change that includes exocrine pancreatic neoplasia, any treatment-related increased incidence of this finding might be evaluated as a potential risk factor for the exocrine pancreas. The lack of difference in acinar hyperplasia between control and treated groups in this study suggests that sitagliptin and metformin do not promote the development of exocrine pancreatic tumors in the rat. Due to the lack of statistical power inherent in comparisons of low-incidence events such as the observed rates for acinar hyperplasia and lobular atrophy in this study, formal testing of PI between foci from control and treated groups was not attempted.

The absence of an increase in PI in the sitagliptin treatment groups in this study is also evidence against the hypothesis that sitagliptin increases the risk of exocrine pancreatic neoplasia by increasing PI. However, this method of assessing increased risk has not been validated (40). The validated, widely used, traditional rodent bioassay is a better established and understood tool for making risk assessments of chemical exposure for human carcinogenic risk. Notably, the mouse and rat bioassays conducted for sitagliptin did not suggest a risk for pancreatic cancer (17).

The absence of pancreatitis in rats treated with sitagliptin in this study with the ZDF rat model of T2DM is consistent with results from previously reported large well-controlled toxicology studies in rodents (17), but differs from reported results in a smaller study of sitagliptin (8). In the HIP rat experiment with sitagliptin, the primary study objective was to evaluate structural and functional effects on the endocrine pancreas, and as part of a functional evaluation of the pancreatic islets, an iv bolus of arginine was administered following a lengthy iv administration of glucose as part of a glucose clamp assessment (8). Intraperitoneal dosing of arginine in the rat is widely used as an experimental model of induced pancreatitis (41), but a dose response for induction of pancreatitis in the HIP rat following bolus iv dosing of arginine has not been reported, making it challenging to interpret the reported pancreatic findings.

In this study, treatment with metformin meaningfully modified the progression of disease in the ZDF rat model (less severe blood glucose changes, increased absolute pancreatic weight, increased body weight, decreased food intake). Accounting for pharmacologically induced changes in disease progression is necessary to make toxicologically relevant comparisons between the metformin group and other groups. In the absence of a control group with an equivalent disease burden, such comparisons were not attempted for the metformin group. In this study, the similarity of the qualitative changes between the metformin group and control groups is consistent with an absence of toxicologically relevant adverse effects on the pancreas, which is also consistent with the historical clinical experience with metformin.

In summary, sitagliptin had no adverse effect on the pancreas of the ZDF rat model of T2DM. There was no evidence of increased risk of pancreatitis, and no difference in exocrine duct and ductular PI between treated and untreated rats.

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