Troglitazone stimulates pancreatic growth in congenitally CCK-A receptor-deficient OLETF rats

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IT IS WELL KNOWN that hypertrophy and hyperplasia of the pancreatic exocrine and endocrine cells in the rat are modified by several metabolic and humoral factors. The gastrointestinal hormone CCK is a well-known stimulus for pancreatic growth such as during a high-protein diet (15, 35), trypsin inhibitor administration (14, 50), diversion of biliopancreatic juice away from the small intestine (44, 55), or repeated subcutaneous injections of the synthetic carboxyl terminal octapeptide of CCK (CCK-8) or caerulein (10, 52). The effect of endogenously released or exogenously administered CCK on pancreatic growth is mediated specifically via CCK-A receptors in the rat (8, 53). The pancreatic endocrine hormone insulin is also a well-known trophic agent that acts on pancreatic exocrine cells (22, 28, 44). In contrast, atrophy of the exocrine pancreas is a characteristic feature of established type 1 diabetes mellitus, where few β cells remain (6, 16).

The Otsuka Long-Evans Tokushima fatty (OLETF) rat is a recently established animal model of diabetes with many similarities to human type 2 diabetes mellitus (20). In this strain of rats, however, the expression of mRNA for the CCK-A receptor in the pancreas, stomach, and brain is completely absent, as confirmed by RT-PCR (12, 37). Although the exocrine pancreas of OLETF rats is totally and specifically insensitive to exogenous and endogenous CCK stimulation (12, 37, 48, 62), the pancreatic wet weight of these rats increases significantly with age (33). Moreover, pancreatic wet weight of OLETF rats treated with the α-glucosidase inhibitor acarbose is significantly heavier than that of untreated OLETF rats (65). These observations suggest that factors other than CCK play important roles in the growth of the pancreas in OLETF rats.

Treatment for type 2 diabetic patients, who have low insulin secretion and resistance to insulin action, consists of reducing hyperglycemia by diet, exercise, or pharmacologically. The recently developed compound troglitazone is reported to improve insulin sensitivity and glucose tolerance by reducing insulin resistance and potentiating insulin action, without stimulating β cell insulin secretion in patients with type 2 diabetes (18, 23, 42). Based on these characteristics, we designed the present study to determine whether diabetic control with troglitazone influences pancreatic growth in genetically CCK-A receptor-deficient, obese, and diabetic OLETF rats (12, 20, 37, 48, 61).

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MATERIALS AND METHODS

Animals and diet. Male OLETF rats, 5 wk of age, were kindly supplied by the Tokushima Research Institute (Otsuka Pharmaceutical, Tokushima, Japan) and were maintained in a temperature (23 ± 2°C) and humidity (55 ± 5%)-controlled room with a 12:12-h light-dark cycle (lights on at 7:00 AM). The rats were provided with humane care according to the guidelines of our institution, and the present experimental protocol was approved by the animal welfare committee. Animals were fed a standard rat chow until the commencement of the experiment at 12 wk of age when one group of rats was used for the control (Cont-12). Next, the OLETF rats were randomly divided into five groups. The first group of rats was given a troglitazone-rich diet (200 mg/100 g normal chow) from 12 (before the onset of diabetes) to 28 wk of age (Tro12–28). The second group of rats was maintained on a troglitazone-rich diet from 12 wk of age until the end of the study at 72 wk (Tro12–72). The third group of rats received a troglitazone-rich chow diet from 28 wk of age (after the onset of diabetes) until the end of the study (Tro28–72). The fourth and fifth groups received standard rat chow free of troglitazone until 28 wk of age or until the end of the study. At the end of the experiments (12, 28, and 72 wk of age) and after a 16-h overnight fast, blood samples were collected for the determination of glucose and insulin concentrations, the rats were killed, and the whole pancreas was removed, cleared of lymph nodes, and weighed. A portion of the pancreatic tissue was homogenized in saline using a motor-driven, Teflon-coated glass homogenizer at 3,000 rpm (8 passes). The homogenates were filtered through three layers of gauze and then sonicated for 1 min. The aqueous phase obtained after 15 min of standing was used for protein, DNA, amylase, lipase, and trypsin assay. Insulin was extracted by a modified method of Davoren (7). Assays. Serum glucose concentrations were determined by the glucose-oxidase method using a glucose kit (Glucose-É reagent; International Reagents, Kobe, Japan; see Ref. 2). Insulin concentrations in the serum and pancreatic homogenates were measured by RIA using the double-antibody method (34) with a commercially available RIA kit (Shionogi RIA; Shionogi Pharmaceutical) using crystalline rat insulin as a reference standard. Protein and DNA concentrations in pancreatic homogenates were determined by the method of Lowry et al. (29) using bovine plasma albumin as a standard and by the method of Labarca and Paigen (24) using the fluorescent dye H-33258 (Hoechst, Frankfurt, Germany) and calf thymus DNA (type I; Sigma Chemical, St. Louis, MO) as a standard, respectively. Amylase activity was determined by a chromogenic method with the Phadebas amylase test (5) and was expressed as a Somogyi unit. Trypsinogen was determined as trypsin activity after activation with enterokinase by the method of Erlanger et al. (9) and was expressed as an N-benzoyl-DL-arginine ethylester hydrochloride unit. Lipase activity was determined by the method of Whitaker (63) and was expressed as International units. Insulin resistance was estimated by the homeostasis model assessment (HOMA) score calculated with the formula fasting insulin (µU/ml) × fasting glucose (mmol/l)/22.5, as described by Matthews et al. (32). With such a method, high HOMA scores denote low insulin sensitivity (insulin resistance). A recent study has demonstrated a strong correlation between clamp-measured total glucose disposal and HOMA-estimated insulin sensitivity (3).

Histological examination. A portion of the pancreatic tissue was fixed overnight in 10% formaldehyde solution for hematoxylin and eosin staining for light microscopic examination. All histological samples were examined by the pathologist in a single-blind fashion without awareness of the treatment modality.

Statistical analysis. Values were expressed as means ± SE. Differences between groups were tested for statistical significance using ANOVA, followed by Tukey’s test. A P value <0.05 denoted the presence of a statistically significant difference.

RESULTS

Serum glucose and insulin concentrations. Fasting serum glucose concentration in untreated control OLETF rats increased progressively with age. Supplementation of the diet with troglitazone almost completely prevented age-related increases in serum glucose concentrations (Fig. 1A). Serum insulin concentrations in control OLETF rats were greatly increased at 28 wk of age but were significantly decreased at 72 wk of age compared with those at 12 and/or 28 wk of age (Fig. 1B). In contrast, serum insulin concentrations in troglitazone-treated rats were nearly the same at all ages and were similar to those in control rats before the onset of diabetes mellitus (Cont-12), irrespective of whether troglitazone was given before (Tro12–72) or after the onset of diabetes (Tro28–72) or given for a relatively short period (Tro12–28). Troglitazone treatment, therefore, significantly lowered fasting serum insulin concentrations at 28 wk of age but increased at 72 wk of age compared with those in the control rats at the corresponding age (Fig. 1B).

Insulin resistance estimated by HOMA scores in control OLETF rats was greatly increased at 28 wk of age compared with at 12 wk of age but was significantly decreased at 72 wk of age and was similar to that at 12 wk of age. In contrast, HOMA scores in troglitazone-treated rats were nearly the same at all ages and were greatly lowered compared with those at 28 wk of age (Fig. 1C). Because a recent study has demonstrated a strong correlation between clamp-measured total glucose disposal and HOMA-estimated insulin sensitivity (3), high HOMA scores denote low insulin sensitivity (insulin resistance). However, HOMA cannot be used in type 1 diabetes such as control OLETF rats at 72 wk of age, although definite proof is lacking (3).

Pancreatic weight. Pancreatic wet weight of control OLETF rats expressed as a total was nearly the same at all ages (Fig. 2A) but decreased age dependently when corrected for body weight (Fig. 2B). Treatment with troglitazone significantly increased pancreatic wet weight irrespective of whether it was given before (Tro12–72) or after the onset of diabetes mellitus (Tro28–72) or whether given for a relatively short period (Tro12–28; Fig. 2A). When related to body weight, however, pancreatic wet weight of troglitazone-treated rats also tended to decrease with age but was significantly greater than that of control rats at the corresponding age (Fig. 2B).

Pancreatic protein, DNA, and enzyme contents. Protein and DNA contents in the control OLETF rat pancreas were nearly the same at all ages (Fig. 3, A and B),
whereas the protein per DNA ratio, an indicator of cellular size, decreased with age (Fig. 3C). Administration of troglitazone, regardless of the treatment groups (Tro12–28, Tro12–72, or Tro28–72), significantly increased not only pancreatic protein and DNA contents but also the protein per DNA ratio compared with control rats (Fig. 3, A–C).

Amylase, lipase, and trypsin contents of the untreated OLETF rat pancreas decreased with age irrespective of whether expressed as a total or presented on protein or DNA content (Table 1). In contrast, these enzymes were nearly the same in the troglitazone-treated OLETF rat irrespective of age (28 or 72 wk of age), irrespective of commencement of treatment (12 or 28 wk of age), and irrespective of the periods of treatment (Tro12–28, 16 wk; Tro12–72, 60 wk; Tro28–72, 44 wk). Thus the pancreatic contents of these three enzymes at 72 wk of age in troglitazone-treated rats were significantly higher than those in the controls at the corresponding age.

Pancreatic insulin contents. Pancreatic insulin contents in the control rats changed in a manner similar to that of serum insulin concentrations [increased at 28 wk but decreased at 72 wk compared with 12 wk of age (Table 1)]. Administration of troglitazone, regardless of the treatment groups (Tro12–28, Tro12–72, or Tro28–72), almost completely ameliorated these alterations of pancreatic insulin content. Insulin content in the pancreas of troglitazone-treated rats, irrespective of the treatment group, was nearly the same as that in the control rats at 12 wk of age (before onset of diabetes mellitus). Thus troglitazone significantly reduced the pancreatic insulin content and concentrations relative to protein or DNA at 28 wk of age but significantly increased at 72 wk of age compared with control rats at the corresponding age (Table 1).

Histological findings. Representative photomicrographs of randomly selected sections of the pancreas taken at 12 (Fig. 4A), 28 (Fig. 4, B and D), and 72 (Fig.
4, 6, C, E, and F) wk of age for different treatment groups are shown using the same magnification. The pancreas of untreated control OLETF rats at 28 wk of age was atrophic, and focal regions of fibrosis, fatty replacement, tubular complexes, and mild-to-moderate infiltration of inflammatory cells, mainly lymphocytes, were seen in several lobules (Fig. 4B). Degeneration and destruction of pancreatic acini and proliferation of tubular complexes further progressed in untreated control OLETF rats at 72 wk of age (Fig. 4C). Troglitazone treatment completely prevented (Tro12–28 (Fig. 4D) and Tro12–72 (Fig. 4E)) or reversed these histological alterations (Tro28–72; Fig. 4F) to those seen in the OLETF rats before the onset of diabetes (12 wk of age; Fig. 4A).

**DISCUSSION**

It is well established that exogenous and endogenous CCK stimulates pancreatic growth in the experimental animal (11, 14, 15, 35, 45, 50, 42, 55). Recent studies have shown that pancreatic growth is mediated specifically by CCK-A receptors in the rat (8, 53); the studies also show that the normal pancreatic growth and growth stimulated by an oral administration of protease inhibitors or by pancreaticobiliary diversion can

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**Table 1. Effect of troglitazone treatment on body and pancreatic wet weight and pancreatic enzyme contents in OLETF rats at 28 and 72 wk of age**

<table>
<thead>
<tr>
<th></th>
<th>28 Wk of Age</th>
<th>72 Wk of Age</th>
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<tr>
<td></td>
<td>Cont-12</td>
<td>Cont-28</td>
</tr>
<tr>
<td>Body wt, g</td>
<td>384 ± 6.5</td>
<td>593 ± 9.3a</td>
</tr>
<tr>
<td>Pancreatic wt, mg/rat</td>
<td>834 ± 26</td>
<td>846 ± 58</td>
</tr>
<tr>
<td>Amylase</td>
<td></td>
<td></td>
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<tr>
<td>10⁶ SU/pancreas</td>
<td>76.9 ± 2.6</td>
<td>63.2 ± 4.5a</td>
</tr>
<tr>
<td>SU/mg protein</td>
<td>635.6 ± 23.0</td>
<td>500.4 ± 72.8</td>
</tr>
<tr>
<td>10⁴ SU/mg DNA</td>
<td>12.3 ± 0.6</td>
<td>10.3 ± 0.8</td>
</tr>
<tr>
<td>Lipase</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10⁶ U/pancreas</td>
<td>6.8 ± 0.3</td>
<td>8.9 ± 0.2a</td>
</tr>
<tr>
<td>U/mg protein</td>
<td>69.9 ± 4.6</td>
<td>49.7 ± 4.5a</td>
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<tr>
<td>10⁴ U/mg DNA</td>
<td>1.54 ± 0.23</td>
<td>1.24 ± 0.28</td>
</tr>
<tr>
<td>Trypsin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10⁶ U/pancreas</td>
<td>63.2 ± 3.2</td>
<td>51.3 ± 2.8</td>
</tr>
<tr>
<td>U/mg protein</td>
<td>668 ± 37</td>
<td>408 ± 49a</td>
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<tr>
<td>10⁴ U/mg DNA</td>
<td>13.1 ± 1.0</td>
<td>8.5 ± 1.0a</td>
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<tr>
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<tr>
<td>nmol/pancreas</td>
<td>22.0 ± 2.4</td>
<td>31.0 ± 0.8a</td>
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<tr>
<td>nmol/mg protein</td>
<td>0.19 ± 0.04</td>
<td>0.23 ± 0.03</td>
</tr>
<tr>
<td>nmol/mg DNA</td>
<td>4.08 ± 0.62</td>
<td>4.79 ± 0.62</td>
</tr>
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Values are means ± SE of 6–8 rats. Tro12-28, troglitazone-rich diet was given from 12 (before the onset of diabetes) to 28 wk of age; Tro12-72, troglitazone-rich diet was given from 12 to 72 wk of age; Tro28-72, troglitazone-rich diet was given from 28 (after the onset of diabetes) to 72 wk of age. In control rats, standard rat chow free of troglitazone was given until 12 (Cont-12), 28 (Cont-28), or 72 (Cont-72) wk of age. SU, Somogyi unit. *Significant difference vs. Cont-12. †Significant difference vs. Cont-28. §Significant difference vs. Cont-72. ‡Significant difference vs. Tro12-28.
completely be inhibited by specific and potent CCK receptor antagonists such as devazepide and loxiglu- midide (38–40, 64). The above studies suggest that CCK plays an important physiological role in pancreatic growth. However, long-term administration of lorglumide, a CCK-A receptor antagonist, for 9 mo failed to further worsen the atrophy of the exocrine pancreas compared with that after a 10-day application of the same substance (40). Moreover, pancreatic weight is found to increase significantly with age, even in the CCK-A receptor-deficient OLETF rats (33). The most recent studies have demonstrated the presence of normal pancreatic weight and cellular morphology in mice lacking functional CCK-A receptors (21) and CCK-deficient mice (25). These results suggest that CCK is a physiological growth factor for the pancreas but it is not an essential one. There must be some important factors other than CCK for pancreatic growth and maintenance of pancreatic function.

Several lines of evidence suggest a regulatory role for insulin in the exocrine pancreas (11, 17, 22, 28, 36, 44, 46, 47, 51). Histological studies have demonstrated that acinar cells adjacent to islets are larger in size and richer in zymogen granules than other acini, showing “haloes” (11). This halo phenomenon is more pronounced in hyperinsulinemic obese mice (17), suggesting that endogenous insulin plays an important role on acinar cell growth and biosynthesis of pancreatic digestive enzymes. Induction of insulinopenic diabetes (type 1) with either alloxan or streptozotocin results in a progressive fall in pancreatic amylase levels and pancreatic weight that can be reversed by in vivo administration of insulin (46, 47, 51). Moreover, insulin treatment after major pancreatectomy enhances early proliferation of the remnant pancreas such as DNA and polyamine synthesis (44). In support of these observations, in vitro studies have clearly demonstrated the direct stimulatory effect of insulin on acinar cell growth and protein and DNA synthesis in mouse and rat pancreatic acinar cells (22, 36). The presence of high-affinity specific receptors for insulin on isolated rat pancreatic acini further supports the importance of

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**Fig. 4.** Representative photomicrographs of the pancreas of untreated control and troglitazone-treated groups at 12, 28, and 72 wk of age. Note the differences in the degree of connective tissue proliferation. A: pancreas of a representative rat from control untreated OLETF rats at 28 wk of age (Cont-28) showing degeneration and destruction of pancreatic acini, proliferation of small ducts, and mild-to-moderate infiltration of inflammatory cells, mainly lymphocytes, with fibrosis. B: pancreas of a representative rat from control untreated OLETF rats at 28 wk of age, the progression of the same histological changes was observed. No histological changes were observed in the pancreas of untreated control OLETF rats at 12 wk (A) and troglitazone-treated rats (D: Tro12-28; E: Tro12-72; F: Tro28-72). Hematoxylin and eosin stain; original magnification ×50.
insulin in the regulation of cellular function in the exocrine pancreas (58). These previous studies strongly suggest that the decrease in pancreatic weight and pancreatic contents of protein, DNA, and enzymes in untreated control OLETF rats at 72 wk of age was not due to a congenital CCK-A receptor deficiency but to the development and deterioration of diabetes mellitus, as has been observed in chemically induced experimental diabetes (46, 47, 50) and type 1 diabetic patients (6, 16).

Troglitazone is known to activate the peroxisome proliferator-activated receptor-γ (PPAR-γ), a member of the nuclear hormone-receptor superfamily, and improve insulin sensitivity and glucose tolerance by reducing insulin resistance via an increase in insulin-stimulated glucose disposal, without stimulating B cell insulin secretion (18, 23, 42). Administration of troglitazone ameliorated the sustained hyperglycemia not by increasing serum insulin levels or pancreatic insulin content but by increasing the action of insulin or decreasing insulin resistance in OLETF rats, which was clearly shown by a marked improvement of the HOMA score (3). Troglitazone decreased both serum insulin concentrations and pancreatic insulin content at 28 wk of age, but it increased them at 72 wk of age compared with those in untreated control OLETF rats at the corresponding age. Thus serum glucose and insulin levels and the pancreatic insulin content in troglitazone-treated rats at 28 and 72 wk of age were similar to those in control rats before the onset of diabetes (Cont-12). Administration of troglitazone stimulated pancreatic growth or prevented pancreatic degeneration when given before the onset of diabetes mellitus (Tro12–28 and Tro12–72) and reversed the degenerated pancreas and enhanced pancreatic growth, even when given after the onset of diabetes mellitus (Tro28–72).Troglitazone administration might have spared B cell overwork by improving insulin sensitivity and thus preventing rats from becoming hyperinsulinemic at 28 wk of age and hypoinsulinemic at 72 wk of age. Troglitazone feeding induced hypotrophy more than hyperplasia and promoted an increase in all of the exportable enzymes. These results are quite different from those in rats treated with trypsin inhibitor or exogenous CCK. Trypsin inhibitor induces hypertrophy more than hyperplasia, as was found in the troglitazone-treated OLETF rats, but it causes nonparallel increases in pancreatic enzyme contents (49). On the other hand, subcutaneous injections of CCK cause a pronounced increase in all of the exportable enzymes but induce hyperplasia more than hypotrophy (52), as in the troglitazone-treated OLETF rats.

Serum insulin concentrations and pancreatic insulin contents in control untreated OLETF rats at 28 wk of age were significantly higher than those in control rats at 12 wk of age (before the onset of diabetes mellitus) and those in troglitazone-treated rats (Tro12–28). In contrast to previous studies that have demonstrated important trophic effects of insulin on the exocrine pancreas (11, 17, 22, 28, 36, 44, 46, 47, 51), our results showed that pancreatic wet weight and pancreatic contents of protein, DNA, and enzymes in untreated control rats at 28 wk of age were significantly lower than those in troglitazone-treated rats (Tro12–28). Because troglitazone reduces insulin resistance and improves insulin sensitivity and glucose tolerance, without stimulating B cell insulin secretion (18, 23, 42), the present data indicate that the important factor for pancreatic growth and maintenance is not insulin level in serum or in the pancreas but diabetic control or improvement of insulin resistance in OLETF rats. In support of this view, improvement of insulin sensitivity by treatment with α-glucosidase inhibitor (65) or by exercise training (59) is shown to have the same effect on the pancreas in OLETF rats.

It appears that pancreatic insulin receptors are involved in mediating the trophic response initiated by endogenous insulin (6, 16, 36, 58). Many target cells can respond to alternations in hormone concentrations by regulating the number of binding sites and/or their affinity for the hormone (4, 13). Receptor occupancy by its ligand leads to a decrease in functional receptor number. If pancreatic growth is stimulated by a sustained increase in serum insulin concentrations, receptor downregulation might damp out the growth response of the pancreas. Indeed, we found in our previous study that amylase output in response to caerulein was significantly lower in the insulin-secretting tumor-bearing than in the control pancreas, although the insulin response to glucose and caerulein was greatly increased (57). In addition, insulin was shown to cause a time- and concentration-dependent reduction in specific receptors for somatomedin/insulin-like growth factor-I (56), which, like insulin, can stimulate cell growth by increasing DNA synthesis and promoting cell differentiation (26, 61). It is possible, therefore, that insulin enhances early pancreatic growth but sustained and excessive insulin reduces the functional number of insulin and somatomedin/insulin-like growth factor-I receptors, resulting in reduced pancreatic growth and fibrosis.

Recent studies have demonstrated that serum leptin concentrations in OLETF rats above the age of 8 wk are three to five times higher than those of their control counterpart Long-Evans Tokushima Otsuka rats, suggesting a decrease in functional leptin receptors (leptin resistance) in OLETF rats (1, 41). Leptin is an adipocyte-derived hormone that belongs structurally to the long-chain helical cytokine family, such as interleukin-2, interleukin-12, and growth hormone, and signals by a class I cytokine receptor (31). Studies of rodents with genetic abnormalities in leptin or leptin receptors revealed obesity-related deficits in macrophage phagocytosis and the expression of proinflammatory cytokines both in vivo and in vitro (27). Indeed, Yang et al. (66) have demonstrated that ob/ob mice exhibit increased sensitivity to endotoxin-induced liver injury and lethality, identifying a potent link between leptin deficiency and the dysregulated expression of endotoxin-inducible cytokines. These previous studies obtained in different strains of genetically obese rodents with a defect at different sites in the leptin-
dependent signaling pathway may provide a pathogenetic mechanism that contributes to focal regions of fibrosis, fatty replacement, tubular complexes, and mild-to-moderate infiltration of inflammatory cells, mainly lymphocytes, in the degenerated exocrine pancreas of untreated control OLETF rats at 28 and 72 wk of age (Fig. 4).

Although PPAR-γ was originally characterized as a regulator of adipocyte differentiation and lipid metabolism (60), recent studies have demonstrated an immunomodulatory role for PPAR-γ in cells critical to the innate immune system, the monocyte/macrophage (19). PPAR-γ inhibits the expression of genes that become upregulated during macrophage differentiation and activation (54) and suppresses monocyte production of inflammatory cytokines (19). Ogawa et al. (43) recently demonstrated that troglitazone treatment prevents multiple, low-dose streptozotocin-induced hyperglycemia by suppressing the infiltration of leukocytes to pancreatic islets (insulitis) and tumor necrosis factor-α production from intraperitoneal exudate cells. The most recent study by Marra et al. (30) has clearly revealed that activation of PPAR-γ by troglitazone results in complete inhibition of hepatic stellate cell proliferation, migration, and expression of monocyte chemotactic protein 1 at the gene and protein levels that contribute to the process of liver inflammation and fibrosis. Consistent with these observations, troglitazone administration in the present study not only prevented but also reversed histological alterations of the pancreas in untreated control OLETF rats, such as derangement of islets, destruction of pancreatic acini, inflammatory cell infiltration, and fibrosis (Fig. 4). Together with an increase in insulin sensitivity, troglitazone might have enhanced pancreatic growth by regulating inflammatory responses in the pancreas of CCK-A receptor-deficient OLETF rats.

**Perspectives**

Administration of troglitazone to the CCK-A receptor-deficient OLETF rats, an animal model for type 2 diabetes mellitus, stimulated pancreatic growth or prevented pancreatic degeneration when given before the onset of histological alterations, such as fibrosis, fatty replacement, tubular complexes, and inflammatory cell infiltration, and reversed the degenerated pancreas and enhanced pancreatic growth when given after the onset of these histological changes. Because atrophy of the pancreas and histological alterations were found irrespective of whether they were hyperinsulinemic or hypoinsulinemic, the important factor for pancreatic growth and maintenance is not insulin level in serum or in the pancreas but diabetic control or improvement of insulin resistance. Moreover, there is a possibility that insulin enhances early pancreatic growth, but a sustained overdose of insulin leads to subsequent fibrosis and reduction of pancreatic growth.

Troglitazone improves insulin sensitivity and glucose tolerance by activating the PPAR-γ, which plays an immunomodulatory role in cells critical to the innate immune system. Troglitazone treatment suppresses the infiltration of leukocytes to pancreatic islets and tumor necrosis factor-α production. Moreover, activation of PPAR-γ by troglitazone results in complete inhibition of hepatic stellate cell proliferation, migration, and expression of monocyte chemotactic protein 1, which contribute to the process of liver inflammation and fibrosis. Troglitazone administration in the present study not only prevented but also reversed histological alterations of the pancreas such as derangement of islets, destruction of pancreatic acini, inflammatory cell infiltration, and fibrosis. Together with an increase in insulin sensitivity, troglitazone might have enhanced pancreatic growth by regulating inflammatory responses in the pancreas of CCK-A receptor-deficient OLETF rats. Although it is difficult to transfer the present observations made in a particular animal model to the human situation, our long-term study in a rodent model of type 2 diabetes mellitus showed that troglitazone can reverse the histopathological alterations in the pancreas such as inflammatory cell infiltration, tubular complexes, and fibrosis, which are identical to those of human chronic pancreatitis. We conclude that anti-inflammatory effects of troglitazone may offer a new therapy for chronic pancreatitis.

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**REFERENCES**


