Case report

Histopathological Changes in the Pancreas from a Spontaneous Hyperglycemic Cynomolgus Monkey

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Abstract: Morphological and immunohistochemical examinations were carried out on the pancreas of a hyperglycemic 5-year-old male cynomolgus monkey. Body weight gradually decreased from 6 months before termination, accompanying a slight reduction in food consumption and anorexia for the last 2 days. The blood glucose level was markedly elevated when examined at termination. Histopathologically, in the exocrine pancreas, diffuse hyperplasia of centroacinar and intercalated duct cells and diffuse atrophy of acinar cells with sporadic apoptosis were observed, although most centroacinar and intercalated duct cells were proliferating cell nuclear antigen (PCNA)-positive in both the present case and age-matched control animals. In the endocrine pancreas, the islets tended to be hypertrophic, with an increase in insulin-positive cells in comparison with the age-matched control animals. PCNA-positive cells also tended to increase in the islets, although positive cells for phospho-histone H3, a marker for mitotic cells, were not detected in the endocrine and exocrine pancreas. Moreover, neither inflammation nor amyloidosis was noted in the islets. In conclusion, the present case probably suffered from early-stage type 2 diabetes mellitus, and it provides fundamental information concerning pancreatic histopathology under insulin-related derangement in monkeys. (DOI: 10.1293/tox.25.215; J Toxicol Pathol 2012; 25: 215–219)

Key words: spontaneous diabetes mellitus, pancreas, β-cell hyperplasia, centroacinar and intercalated duct cell hyperplasia, cynomolgus monkey

Diabetes mellitus (DM) is characterized by persistent hyperglycemia due to defects in insulin production, secretion or action and is roughly divided into type 1 and type 2 DM. Type 1 DM is primarily brought about by destruction of β-cells due to a polygenic autoimmune response, resulting in a decrease in the number and size of islets. Type 1 DM is likely to occur at a young age, while type 2 DM, which is caused by insulin resistance in target tissues, commonly develops at an adult age. In the early stage of type 2 DM, proliferation of β-cells is one of the characteristic findings, and this seems to be a compensatory response to hyperglycemia in order to maintain euglycemia. Such proliferation of β-cells leads not only to islet hypertrophy but also to amyloid deposition, since β-cells can produce an amyloid peptide, amylin. However, the cellularity decreases along with abundant amyloid deposition in the islets of advanced type 2 DM. In a survey of nonhuman primates with DM, all animals examined had type 2 DM with amyloidosis in the islets. The clinicopathologic characterization of spontaneous DM in vervet monkeys was well documented by Cann et al. In cynomolgus monkeys, the natural occurrence of type 2 DM is higher than that of type 1 DM. The present paper describes the histopathological and immunohistochemical features of the pancreas in a young cynomolgus monkey that probably suffered from early-stage type 2 DM.

The animal was a 5-year-old male cynomolgus monkey and was a spare animal for toxicological studies (Hainan Jingang Laboratory Animal Co., Ltd., Hainan Province, China). Behavioral and clinical tests had not been done on the animal except for measurement of body weight and food consumption at a several time points before termination. This animal was housed alone in a stainless steel cage (W730 × D720 × H800 mm) in an animal room maintained under controlled conditions (temperature, 21 ± 5°C; relative humidity, 55 ± 15%; air ventilation 8 to 10 times per hour; artificial lighting, 12-hour light/12-hour dark cycle), was supplied 150 g of pellet diet for monkeys (carbohydrate, protein and fat concentration: 52, 23 and 8%, SLACOM® SLAC-MK01, SLAC Laboratory Animal Co., Ltd., Shanghai, China) in the afternoon and also 50 g of fruits or vegetable in the morning and was allowed free access to tap water. The animal was cared for according to the principles outlined in the Regulations for the Administration of Affairs Concerning Experimental Animals, Decree No.2, approved

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by the State Council of the People’s Republic of China, 1988 and the Regulations for the Administration of Affairs Concerning Experimental Animals Approved by the Zhejiang Provincial Government in 2009.

The animal showed no distinct abnormal clinical signs, except for a gradual decrease in body weight, from 4.9 kg at 6 months before sacrifice in moribund condition to 4.0 kg at termination (5.75 ± 1.11 kg with a range of 3.6 to 7.7 kg in our background data), and a slight loss of appetite and anorexia for the last 2 days. No data suggestive of obesity were recorded prior to development of the disease. Clinico-pathological examinations done at termination revealed that the blood glucose level had markedly elevated to 565.5 mg/dL (82 ± 18 mg/dL with a range of 23 to 208 mg/dL in our background data), while the serum levels of triglycerides and total cholesterol were nearly within normal values (35 mg/dL and 168 mg/dL, respectively) (30 ± 17 mg/dL and 117 ± 26 mg/dL in our background data, respectively). No abnormal macroscopic changes were noted. After a complete necropsy, all tissues were preserved in 10% neutral-buffered formalin and then embedded in paraffin. As for the pancreas, the tissue was obtained from its tail part. Thin sections from all tissues were stained with hematoxylin and eosin (HE). To assess the islet size and number, morphometric analysis was conducted using a Luezex AP image processor (Nireko Corporation, Tokyo, Japan). In addition, additional sections of the pancreas were also subjected to Congo red staining for amyloid detection under polarized lens and immunohistochemistry for insulin (monoclonal, Z006, Nichirei Biosciences, Tokyo, Japan), proliferating cell nuclear antigen (PCNA) (monoclonal, PC10, 1:5000, DakoCytomation, Glostrup, Denmark), phospho-histone H3 (polyclonal, 1:150, Cell Signaling Technology, Beverly, MA, USA), a marker for cell division, and cleaved caspase-3 (CASP3) (polyclonal, 1:200, Cell Signaling Technology), a marker for apoptosis, using an Envision® kit (DAKO Japan, Tokyo, polyclonal, 1:200, Cell Signaling Technology), a marker for cell division, and cleaved caspase-3 (CASP3) (polyclonal, 1:200, Cell Signaling Technology), a marker for apoptosis, using an Envision® kit (DAKO Japan, Tokyo, Japan). For electron microscopic examination, small pieces of the formalin-fixed pancreatic tissues were fixed with 0.5% glutaraldehyde and 1.5% paraformaldehyde, postfixed with 1% osmium tetroxide and embedded in epoxy resin (OkenShoji Co., Ltd., Tokyo, Japan). Ultrathin sections were stained with uranyl acetate and lead citrate and were examined under a transmission electron microscope. For the sake of comparison to the present case, the pancreases from 3 age-matched normal male cynomolgus monkeys were also examined in the same way as control animals.

Histopathologically, in addition to the pancreatic changes mentioned below, focal pneumonia, vacuolation of renal tubule epithelial cells, adenocortical hypertrophy, lymphoid depletion in various lymphoid tissues and gastritis were observed minimally or mildly. These changes were thought to be secondary to the animal’s poor general condition and/or anorexia for 2 days before termination.

In the exocrine pancreas, diffuse proliferation of centroacinar and intercalated duct cells and diffuse atrophy of acinar cells (Figs. 1a–1d) with sporadic pyknotic and CAS3-positive acinar cells were noted (Fig. 2g), although most centroacinar and intercalated duct cells were PCNA positive in both the present case and the comparative animals (Figs. 2e and 2f).

In the endocrine pancreas, the islets tended to be enlarged in comparison with the control animals, although there was no distinct difference in the number of islets (Figs. 1a and 1c, Table 1). Immunohistochemically, compared with control animals, the number of insulin-positive cells increased apparently in the central area of islets (Figs. 2a–2d), where glucagon-positive cells are usually the predominant component cells in normal monkeys. In addition, the number of PCNA-positive cells was slightly increased mainly in the peripheral area of islets as compared with the control animals. At least a part of such PCNA-positive cells may be β-cells. On the other hand, no phospho-histone H3-positive cells were detected in the endocrine and exocrine pancreas of any animals. In the islets, neither amyloid deposition nor inflammatory cell infiltration was noted in the islets, and there were no abnormal ultrastructural findings detected in the component cells (Fig. 3).

Previous investigators demonstrated the differences in pancreatic histology and immunohistochemistry between type 1 DM and type 2 DM in monkeys

| Table 1. Morphometric Analysis of Islets in the Present Case and the Age-matched Control Animals |
|----------------------------------------------------------|
| Islet size (mm²) | Number of islets |
|------------------|------------------|
|                  | Mean ± SD        | Maximum |
| Present case     | 0.022±0.024      | 0.111   |
| Age-matched control animals |
| #1               | 0.015 ± 0.012    | 0.067   |
| #2               | 0.017 ± 0.016    | 0.103   |
| #3               | 0.014 ± 0.012    | 0.064   |

a) The islets more than 0.005 mm² were measured in one section of the pancreatic tail per animal.
amyloid deposition in the present case. In addition to such histological and immunohistochemical similarities, marked hyperglycemia and absence of DM-related complications further supported that the present case might be early-stage type 2 DM. On the other hand, the continuous decrease in body weight observed in the present case is not a general finding of early-stage type 2 DM and may not be related to the diabetic condition. Judging from the above-mentioned histological, ultrastructural and immunohistochemical findings of β-cells, the hyperglycemia observed in the present case might be related to a defect in insulin action or to insulin resistance in target tissues, not to a defect in insulin production, although blood insulin concentrations were not measured in the present study.

It is well known that islet cells have a potential to proliferate and regenerate themselves. Experimentally, proliferation of β-cells has been reported following such treatments as cellophane wrapping of the pancreas, partial pancreatectomy, ductal ligation and streptozotocin injection. Kim et al. demonstrated that insulin-positive cells appeared in the acini and intercalated ducts in streptozotocin-induced diabetes in rats. Similarly, proliferation of ductuloendocrine cells was demonstrated in young dogs with spontaneous DM. β-cell neogenesis is a matter of concern, especially in regeneration medicine for DM. To date, intra-islet precursor cells, acinar and ductal cells, stem cells in the ducts and transdifferentiated acinar and/or duct cells have been suggested as possible precursors...
of islets. In the present case, although there was no direct evidence of islet neogenesis, it was suggested that the preexisting β-cells in the islets might slowly proliferate.

As mentioned above, diffuse proliferation of centroacinar and intercalated duct cells was one of the discriminatory findings in the present case. Poor1 demonstrated a highly proliferative activity in centroacinar cells in insular regeneration and speculated that the centroacinar cells and intercalated duct cells may have potential as stem cells. In the present case, diffuse proliferation of centroacinar and intercalated duct cells could be a slowly developing reactive change that was probably initiated a long time ago, since no specific cells for phospho-histone H3, a marker for cell division, were detected. On the other hand, diffuse atrophy by sporadic appearance of apoptosis in acinar cells may be related to deterioration and/or anorexia, since it was associated with no degenerative changes.

In conclusion, the present case was considered to be young-onset type 2 DM in a monkey with diffuse hyperplasia of centroacinar and intercalated duct cells, which has not been described in the previous reports of DM. Further studies are needed to clarify the meaning of the hyperplastic changes observed in the present case. DM is one of the highly prevalent human diseases, and antidiabetic drugs are now being actively developed. The present report may contribute to assessment of histopathological changes of the pancreas under insulin or blood glucose derangement induced by chemical treatments.

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