Inhibitory Effects of Octreotide Acetate, a Somatostatin Analog, on Spontaneous Chronic Pancreatitis in WBN/Kob Rats

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Abstract: Effects of octreotide acetate, a somatostatin analog, on the development of spontaneous pancreatitis were investigated in WBN/Kob rats. Delivery of the agent continuously for 28 days via osmotic pumps implanted subcutaneously at 6 µg/day (group 1), 3 µg/day (group 2) or 0 µg/day (saline) (group 3) resulted in comparable body weight gain in all three groups. Relative weights of the liver, kidney, testis, spleen and pancreas also did not significantly differ between the treatments. Blood glucose levels were lowered by the high, but not the low dose treatment, while plasma somatostatin levels were remarkably increased in both the octreotide treatment groups. Remarkable hemorrhage, inflammatory cell infiltration, fibrosis, vacuolation of acinar cells and ductular proliferation were observed in the pancreas of control rats in group 3. However, these findings were consistently less intense in the octreotide treatment groups, in line with morphometric data showing fibrotic areas to be significantly (P<0.01) reduced.

Immunohistochemically, collagen fibers in the intralobular space were mainly of type-III and mixed with α-smooth muscle actin, reflecting fibrosis in all groups. The present experiment demonstrated that octreotide inhibits spontaneous pancreatitis in WBN/Kob rats. (J Toxicol Pathol 2007; 20: 71–75)

Key words: WBN/Kob rat, pancreatitis, octreotide acetate

Introduction

The Wistar rat strain Bonn/Kobori (WBN/Kob) first originated in a colony at the Institute of Experimental Gerontology in Basel and has been inbred by sister-brother mating at the Institute of Pathology, University of Bonn since 19651. This strain is known to be susceptible to experimental tumor induction in the glandular stomach, especially in the pyloric region1. It has been well documented that male Wistar WBN/Kob rats spontaneously develop pancreatitis when they are young, and also suffer from a diabetic syndrome before 1 year of age2. The pancreatic lesions are characterized by the destruction of not only B but also A cells, accompanied by pancreatic insufficiency due to fibrous replacement of the exocrine pancreas. Thus, the pathophysiology of diabetes in WBN/Kob rats is quite different from that of other previously reported diabetic animal models such as NOD mice, BB rats and KK mice2. Briefly, the characteristic features are (a) gradual onset of symptoms with polyuria, polydipsia, and weight loss accompanied by glycosuria and hyperglycemia; (b) insulin deficiency with a remarkable decrease in the number and size of islets, and decreased insulin content of pancreatic tissue; and (c) multifocal fibrosis with exocrine pancreatic insufficiency2.

Somatostatin has been found to be present in many tissues of the body of both humans and experimental animals, but the gastrointestinal tract contains >70% of the total body somatostatin with particularly high concentrations in the stomach and pancreas3. This hormone inhibits secretion of saliva, gastric acid, pepsin, intrinsic factor and increases gastric mucus production4. In the pancreas, it inhibits the secretion of digestive enzymes and bicarbonate, and reduces the volume of pancreatic juice, although the effects vary with the doses administered and in extreme cases it can inversely stimulate pancreatic function5.

Octreotide acetate is a somatostatin analog with a plasma half-life of 45 min, much greater than the 3 min reported for intrinsic somatostatin in humans6. It is a more potent inhibitor of growth hormone, glucagon, insulin and gastric acid secretion than somatostatin and has been shown to prevent the development of experimentally induced acute pancreatitis in rats2, although it promoted hamster pancreatic...
carcinogenesis when administered at a low dosage. In the present study, the effects of octreotide on the development of spontaneous pancreatitis were evaluated in WBN/Kob rats.

**Materials and Methods**

**Animals and chemicals**

A total of 50 male WBN/Kob rats (Japan SLC, Inc., Shizuoka, Japan), 10 weeks old and weighing about 250 g at the commencement, were used in the experiment. The animals were housed, five per polycarbonate cage, in an air-conditioned room at 23 ± 2°C, 60 ± 5% humidity under a daily cycle of alternating 12-h periods of light and darkness. Oriental MF pellet diet (Oriental Yeast Co., Ltd., Tokyo, Japan) and tap water were available ad libitum. Octreotide acetate was obtained from Novartis Pharma K.K. (Tokyo, Japan).

**Animal treatment**

Group 1, 2 and 3 rats, consisting of 20, 20 and 10 animals, respectively, received octreotide acetate delivered continuously for 28 days, via 2ML4 Alzet osmotic pumps (Alzet Corporation, CA, USA) implanted subcutaneously, at 6 µg/day (group 1), 3 µg/day (group 2) or 0 µg/day (saline) (group 3). The rats were observed daily and weighed once every week. At the end of day 28, all surviving animals were sacrificed to allow sampling of pancreas, liver, lung and kidney tissues for histopathological and immunohistochemical examinations. At autopsy the pancreas was carefully removed together with the duodenum and spleen, and extended on filter paper. Each pancreas was divided into the 4 anatomical lobes, i.e., splenic, gastric, duodenal and head.

**Histological and immunohistochemical examinations**

The tissues were fixed in 10% neutral-buffered formalin, and then routinely processed for paraffin embedding and histopathology, as well as immunohistochemistry. Sections, cut at 3 µm, were stained with hematoxylin and eosin (H-E), and azocarmine and additional aniline blue (Azan) for histopathological examination. Azan stained (blue) portions of fibrotic tissue in the pancreas in groups 1, 2 and 3 were also morphometrically analyzed with the aid of an image analyzer IPAP (Sumitomo Technos, Osaka, Japan). For immunohistochemical staining, antibodies against type-I or -III collagen (Biodesign International, Kennebunk, ME), α-smooth muscle actin, insulin and somatostatin (Dako Cytomation, Glostrup, Denmark) were applied as the primary antibodies. Immunostaining was performed, using the streptavidin-biotin complex technique (LSAB2 Universal Kit; Dako Cytomation).

**Analysis of plasma somatostatin levels and blood glucose levels**

Blood samples from all animals were collected from the aorta under ether anesthesia at sacrifice and plasma was frozen at −80°C until analysis for somatostatin levels according to the method described previously. Briefly, somatostatin levels were determined by 125I-labeled radioimmunoassay using a rabbit polyclonal antibody which recognizes both octreotide and intrinsic somatostatin. Blood glucose levels were analyzed with Glucoster and Glucostix (Miles Laboratory Inc., IN, USA).

**Statistical evaluation**

The results were statistically analyzed by analysis of variance (ANOVA) and the Fisher’s exact probability test.

**Results**

**Body and organ weights**

Body weight gain in groups 1 and 2 did not significantly differ from that in group 3 (Fig. 1). Relative weights of the liver, kidney, testis and spleen (Table 1), and pancreas also did not show any significant intergroup variation (Fig. 2).

**Levels of blood glucose and plasma somatostatin**

The average blood glucose value was significantly lower in group 1 (134 ± 33 mg/ml, \( P < 0.05 \)), but the value in group 2 (155 ± 26 mg/ml) was not significantly different than in group 3 (155 ± 25 mg/ml) (Fig. 2). Plasma somatostatin levels were significantly increased in both groups 1 (1277 ± 362 pg/ml, \( P < 0.01 \)) and 2 (435 ± 153 pg/ml, \( P < 0.05 \)) as compared to group 3 (<4.87 pg/ml) in a clear dose-related manner (Fig. 2).

**Histological evaluation**

Remarkable hemorrhage, inflammatory cell infiltration, fibrosis, vacuolation of acinar cells and ductular proliferation were observed in the pancreas tissues of rats in group 3. Most fibrotic areas were nodular and within lobules, involving atrophic islets and proliferating ducts. Interlobular fibrosis was also apparent in some areas (Figs. 3A and 3B). Atrophy of acinar cells was characterized by...
reduction in size, depletion of zymogen granules, scattered single cell necrosis and replacement by duct-like cells. The remaining acini were small and consisted of reduced numbers of acinar cells. A few normal acinar cells with transitional structures, small atrophic acinar cells and cuboidal cells morphologically similar to duct cells were also present. Such pancreatic findings were much less frequent, showing almost normal appearance except slight and focal fibrosis, in rats of groups 1 and 2 (Figs. 3C and 3D). Moreover, alteration of the component cells in the islet was not evident in H-E stained tissue preparations.

Results of morphometrical analysis with an IPAP image analyzer revealed significant decreases in groups 1 (2.7 ± 5.3%, $P<0.05$) and 2 (18.1 ± 17.5%, $P<0.05$) as compared to the area of fibrosis in group 3 (22.8 ± 22.2%), in a dose-related manner (Fig. 2).

**Table 1.** Body and Relative Organ Weights of WBN/Kob Rats Treated with Octreotide

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of rats</th>
<th>Body weight (g)</th>
<th>Liver (g%)</th>
<th>Kidneys (g%)</th>
<th>Testes (g%)</th>
<th>Spleen (g%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. 6 µg/day</td>
<td>20</td>
<td>316 ± 26</td>
<td>2.67 ± 0.13</td>
<td>0.56 ± 0.03</td>
<td>1.25 ± 0.23</td>
<td>0.15 ± 0.02</td>
</tr>
<tr>
<td>2. 3 µg/day</td>
<td>20</td>
<td>320 ± 35</td>
<td>2.70 ± 0.12</td>
<td>0.58 ± 0.03</td>
<td>1.06 ± 0.13</td>
<td>0.16 ± 0.02</td>
</tr>
<tr>
<td>3. 0 µg/day</td>
<td>10</td>
<td>331 ± 46</td>
<td>2.68 ± 0.07</td>
<td>0.61 ± 0.03</td>
<td>1.12 ± 0.19</td>
<td>0.16 ± 0.02</td>
</tr>
</tbody>
</table>

Data represent mean values ± SD.

**Discussion**

The present experiment demonstrated that octreotide inhibits the spontaneous development of pancreatitis in WBN/Kob rats. Blood glucose levels and pancreatic fibrotic areas were remarkably decreased in the high dose octreotide treatment group, and findings for hemorrhage, inflammatory cell infiltration, fibrosis, vacuolation of acinar cells and ductular proliferation were also reduced, although pancreatitis was not completely prevented. It was earlier reported that a protease inhibitor and estrogen inhibited spontaneous pancreatitis in WBN/Kob rats, but this is the first such demonstration for a somatostatin analog.
However, it has been shown that octreotide prevents acute pancreatitis in the rat, possibly by stimulating the reticuloendothelial system, or reducing endotoxaemia. These mechanisms might also have been operating in the present study.

Cerulein-induced and ethionine-induced hemorrhagic pancreatitis, and several other models with ligated common ducts or closed duodenal loops are all available as animal models of acute pancreatitis. However, the pancreas of the WBN/Kob rat with spontaneous pancreatitis demonstrates changes very similar to those in human chronic pancreatitis. Although it has been shown that somatostatin prevents acute pancreatitis, there have hitherto been no reports regarding its effects on chronic pancreatitis.

In WBN/Kob rats fed diet containing a protease inhibitor, the pancreas becomes hypertrophic without any histological evidence of chronic pancreatitis, and the exocrine function is maintained with an increase in plasma cholecystokinin concentrations. The sex specificity observed for development of pancreatic lesions suggests the influence of sex hormones and Nakama et al. reported that subcutaneous injection of estradiol prevented the development of pathologic fibrotic lesions and clinical diabetes. Castration of male WBN/Kob rats is also relatively effective for the prevention of pancreatitis. On the other hand, ovariectomy caused pancreatic lesions in female WBN/Kob rats. Estrogen may regulate the growth of ductular epithelium, exert an anti-inflammatory effect, prevent pancreatic fibrosis, or stimulate the growth of acinar cells. Although the pathogenesis and mechanisms underlying the pancreatic changes occurring in WBN/Kob rats remain to be clarified, the limitation to males and age-dependent onset strongly suggest that certain sex hormones are involved in the etiology of pancreatitis in this strain.

Octreotide exerts inhibitory effects on various aspects of pancreatic function including secretion of insulin and glucagon. It inhibits the secretion of digestive enzymes and bicarbonate, and reduces the volume of pancreatic juice, although these effects vary with the doses administered and in extreme cases it can inversely aggravate pancreatitis secondarily. Histopathologic features of the WBN/Kob rat model are partially similar to those of chronic pancreatitis in humans, which is characterized by massive destruction of both endocrine and exocrine pancreatic tissues and replacement with fibrous deposits.

Mori et al. reported immunohistochemical staining for insulin and glucagon to be decreased with regard to numbers of not only B but also A cells in WBN/Kob rats, in line with their insulin and glucagon contents in comparison with Wistar rats. In the present study, somatostatin-positive cells in large-sized islets tended to be reduced with the octreotide treatment. Although glucagon was not examined, it is likely that there was little change in the type of distribution of islet endocrine cells as a result of the octreotide administration. In conclusion, the results of the present study clearly indicate that a long-acting somatostatin analog, octreotide, inhibits spontaneous pancreatitis in WBN/Kob rats.
References


