Different Effects of Oral Administration of Synthetic Trypsin Inhibitor on the Pancreas Between Cholecystokinin-A Receptor Gene Knockout Mice and Wild Type Mice

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ABSTRACT—The synthetic trypsin inhibitor camostat has been used for the treatment of acute and chronic pancreatitis in Japan based on the evidences obtained from a rat experimental model. However, rats differ from other rodents and from humans in terms of lacking a gallbladder and no response of pancreatic bicarbonate secretion to cholecystokinin (CCK). In the present study, we determined whether oral administration of camostat showed a trophic effect in mice as observed in rats and whether the trophic effect, if substantial, was mediated via the CCK-A receptor, using CCK-A receptor gene targeting mice. The chow containing 0.1% camostat was fed to 8-month-old mice. Three- and seven-day treatments with camostat did not affect pancreatic wet weight in CCK-A receptor (+/+/G2d/G2d) mice. After 14-day treatment, the ratio of pancreatic wet weight/body weight was significantly lower in CCK-A receptor (+/G2d/G2d) than (++/+) mice. The protein and chymotrypsin contents were lower and amylase content was higher in CCK-A receptor (+/G2d/G2d) mice, compared to (++/+) mice. No pathological findings were observed by histological examination. Camostat has a trophic effect on the pancreas in mice and this effect is mediated via the CCK-A receptor, but is less potent than in rats.

Keywords: Cholecystokinin (CCK), CCK-A receptor, Pancreas, Trypsin inhibitor

Cholecystokinin (CCK) is an important biological peptide that induces gallbladder contraction and stimulates pancreatic enzyme secretion (1). It is well known in rats that CCK has a trophic effect on the pancreas and that oral administration of trypsin inhibitor induces pancreatic hypertrophy and hyperplasia via releasing CCK (2, 3). A specific regulatory mechanism of pancreatic exocrine secretion, the so-called luminal feedback regulation, has been demonstrated in conscious rats (4, 5). That is, when luminal protease activity decreased below the threshold (<10%), CCK was released from the endocrine cells in the small intestine (5, 6). Thus, oral administration of trypsin inhibitors decreased luminal trypsin activity, increased CCK release, and produced hyperplasia and hypertrophy of pancreatic acinar cells via CCK-A receptor function in rats (2, 3, 7 – 12).

A synthetic trypsin inhibitor camostat has been used for the treatment of acute and chronic pancreatitis in Japan. Based on the evidences in rats (2, 3, 7 – 12), camostat had been anticipated to enhance regeneration of pancreatic acinar cells as well as to inactivate enzymes. However, recently, the CCK-A receptor was cloned and the differences in the expression of CCK receptors have been reported (13). The human pancreas does not express CCK-A receptor, while it is detected in the gallbladder...
Although the pancreas in mice, rats, and guinea pigs expresses CCK-A receptor gene and protein, rats differ from mice and guinea pigs. Rats do not have a gallbladder and CCK failed to stimulate pancreatic bicarbonate secretion (14–16). Recently, Lacourse et al. reported using CCK gene knockout mice (17) that pancreatic adaptation to oral administration of a high protein diet in terms of pancreatic wet weight, protein content, or enzyme compositions, were similarly observed in wild type [CCK(+/+)] and (−/−) mice. They concluded that pancreatic adaptation to dietary protein does not require CCK in mice (17), although in rats, CCK is the major factor responsible for dietary protein induced pancreatic growth (18–20). Therefore, in the present study, we determined whether oral administration of camostat showed a trophic effect in mice as observed in rats. Moreover, we used CCK-A receptor knockout mice to examine whether the trophic effect of camostat, if it exists, was mediated by the CCK-A receptor. We recently generated CCK-AR gene knockout (−/−) mice and confirmed that CCK had no stimulatory effect on pancreatic amylase secretion in CCK-AR(−/−) mice (16, 21). The pancreas in CCK-AR(−/−) mice could respond to other stimulants such as neuromedin C and acetylcholine, and amylase secretion in response to various stimulants in CCK-AR(+/−) mice were similar to that in CCK-AR(+/+) mice (16, 21). The body weight and the ratio of pancreatic wet weight/body weight were not different among the three genotypes (16).

MATERIALS AND METHODS

Chemicals

A chow containing 0.1% camostat, N,N-dimethylcarbamoylmethyl-p-(p-guanidinobenzoxyloxy) phenylacetate monomethanesulfonate was supplied by Ono Pharmaceutical Co., (Osaka). A commercial chow CRF-1 (Oriental Yeast Co., Tokyo) was used as a control.

Animals

The protocol was reviewed and approved by the appropriate committee of the Tokyo Metropolitan Institute of Gerontology.

Male CCK-AR(+/−) mice bred with female CCK-AR(+/−) mice, and the age-matched (8-month-old) male and female progeny of CCK-AR(+/+), (+/−) and (−/−) mice were used for experiment. The CCK-AR genotype distribution follows the Mendelian rule (16, 21). Thus, more numbers of CCK-AR(+/−) mice were born than CCK-AR(+/+) or (−/−) mice. Moreover, since the amylase secretion in response to CCK, neuromedin C, and acetylcholine in CCK-AR(+/−) mice have been similar to that in CCK-AR(+/+) mice (16, 21), age-matched CCK-AR(+/−) mice were used to determine the treatment period.

Experimental procedures

Determination of treatment period: Control and camostat-containing diets were administered for 0, 3, and 7 days to CCK-AR(+/−) female mice. The mice were sacrificed by guillotine and the pancreas was removed, and weighed. The effect of 14-day treatment with camostat was examined using CCK-AR(+/−) male mice. CCK-AR(+/−) male mice were also used as a control for comparison. The body weight and the ratio of pancreatic wet weight/body weight were recorded.

Effects of 14-day camostat treatment: We found that the ratio of pancreatic wet weight/body weight was significantly increased in CCK-AR(+/−) male mice after 14-day treatment with camostat (Fig. 1). Therefore, to determine the involvement of CCK-AR, age matched male CCK-AR(+/+) and (−/−) mice were prepared and a camostat containing diet was fed for 14 days to these mice. After
14-day treatment, mice were sacrificed by guillotine. The whole pancreas was removed and weighed. Some of specimens were promptly frozen in liquid nitrogen, and lyophilized to measure protein and enzyme concentrations. The rest of the pancreas specimens were immersed in 10% formaldehyde solution for histological examination (HE staining).

**Assays:** Samples of 5 – 10 mg of lyophilized pancreatic tissue were weighed and homogenized in 2 ml of 0.1 M Tris buffer (pH 8.0) containing 0.02 M CaCl₂ and 1% Triton X-100 (3). The protein concentrations were determined by the method of Lowry et al. (22). Amylase activities were measured using a blue starch polymer as a substrate and the porcine amylase (884 u/mg protein) (Worthington, Freehold, NJ, USA) was used as a standard (3). Chymotrypsin activity was assayed by spectrophotometric methods, using N-benzoyl-L-tyrosine ethyl ester (BAEE) as a substrate and purified bovine chymotrypsin (50 u/mg protein) (Worthington) was used as a standard.

**Statistical analyses**

Values are expressed as the mean ± S.E.M. Significance of differences was assessed by one way analysis of variance (ANOVA). A value of P<0.05 was considered significant.

**RESULTS**

**Determination of treatment period of camostat in CCK-AR(+/-) mice**

Body weight, pancreatic wet weight, or the ratio of pancreatic wet weight/body weight did not differ significantly between animals treated with camostat and control diets at any point during the first 7-day treatment. The ratios of pancreatic wet weight/body weight (mg/g) are shown in Fig. 1. After 14-day treatment, the body weight was significantly lower in CCK-AR(+) male mice treated with camostat than those treated with a control diet. The pancreatic wet weight after 14-day treatment with camostat tended to increase, although it was not statistically significant. The ratio of pancreatic wet weight/body weight (mg/g) was significantly increased by 14-day camostat treatment (Fig. 1). The mean value of daily food intake was 2 – 3 g/mice per day and was not significantly different throughout the experimental period.

**Effects of 14-day treatment with camostat in CCK-AR(+/+) and (-/-) mice**

The mean initial body weight of CCK-AR(-/-) mice was significantly higher than that of CCK-AR(+/+) mice [29.9 – 49.0 g for CCK-AR(+/+) mice, and 40.4 – 48.2 g for CCK-AR(-/-), respectively]. Administration of camostat for 14 days significantly decreased body weight in both genotypes, but the percent decrease was not different (86.4% and 88.1% decrease for CCK-AR(+/+) and (-/-) mice, respectively) (Table 1). After 14-day camostat treatment, although the body weight was lower in CCK-AR(+/+) than AR(-/-) mice, the pancreatic wet weight was significantly higher in CCK-AR(+/+) than (-/-) mice (Table 1). When expressed as pancreas wet weight per body weight (mg/g), it was significantly higher in CCK-AR(+/+) than (-/-) mice. The value of CCK-AR(-/-) mice was similar to those of CCK-AR(+/+) mice treated with a control diet shown in Fig. 1, open columns.

The tissue concentrations of protein and chymotrypsin, when expressed as mg/g pancreatic wet weight, were similar to each genotype (Table 2). However, as the pancreatic wet weight was lower in CCK-AR(-/-) mice, the total contents of protein and chymotrypsin in the whole pancreas were significantly higher in CCK-AR(+/+) than (-/-) mice. On the contrary, the amylase concentration (mg/g) was

<p>| Table 1. Changes in body weight, and pancreatic wet weight after 14-day treatment with camostat |</p>
<table>
<thead>
<tr>
<th>Genotype</th>
<th>CCK-AR(+/+)</th>
<th>CCK-AR(-/-)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 10)</td>
<td>(n = 4)</td>
</tr>
<tr>
<td>Initial body weight (g)</td>
<td>33.2 ± 1.5*</td>
<td>44.8 ± 1.4</td>
</tr>
<tr>
<td>Body weight after 14-day treatment (g)</td>
<td>28.7 ± 1.3*</td>
<td>39.5 ± 1.9</td>
</tr>
<tr>
<td>Body weight decrement (%)</td>
<td>86.4%</td>
<td>88.1%</td>
</tr>
<tr>
<td>Pancreatic wet weight (mg)</td>
<td>522 ± 24*</td>
<td>299 ± 69</td>
</tr>
<tr>
<td>Pancreatic wet weight /Body weight (mg/g)</td>
<td>18.6 ± 1.3*</td>
<td>7.5 ± 1.4</td>
</tr>
</tbody>
</table>

Values are the mean ± S.E.M. Age-matched male mice were used. *Significantly different from the corresponding values. The numbers in the parentheses are the numbers of animals.

<p>| Table 2. Protein, chymotrypsin and amylase contents in CCK-AR(+/+) and (-/-) mice after 14-day treatment with camostat |</p>
<table>
<thead>
<tr>
<th>Genotype</th>
<th>CCK-AR(+/+)</th>
<th>CCK-AR(-/-)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein concentration (mg/g pancreas)</td>
<td>370.5 ± 11.9</td>
<td>366.7 ± 32.3</td>
</tr>
<tr>
<td>Protein content in the whole tissue (mg)</td>
<td>194.6 ± 13.1*</td>
<td>115.2 ± 37.3</td>
</tr>
<tr>
<td>Chymotrypsin concentration (mg/g pancreas)</td>
<td>1.37 ± 0.10</td>
<td>0.94 ± 0.40</td>
</tr>
<tr>
<td>Chymotrypsin content in the whole tissue (mg)</td>
<td>0.73 ± 0.07*</td>
<td>0.36 ± 0.24</td>
</tr>
<tr>
<td>Amylase concentration (mg/g pancreas)</td>
<td>16.67 ± 3.7*</td>
<td>40.04 ± 11.8</td>
</tr>
<tr>
<td>Amylase content in the whole tissue (mg)</td>
<td>8.4 ± 1.2</td>
<td>12.4 ± 2.5</td>
</tr>
</tbody>
</table>

Values are the mean ± S.E.M. Age matched male mice were used. *Significantly different from the corresponding values. The numbers of animals are shown in Table 1.
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significantly lower in CCK-AR(+/+) than (−/−) mice. However, the total content tended to be lower in CCK-AR(+/+) mice than (−/−) mice, but were not statistically significant.

Histology

Histologic examination revealed no pathological findings, and there were no significant differences between CCK-AR(+/+) and (−/−) mice with or without camostat (Fig. 2). For the comparison, the previously obtained pancreatic tissues from CCK-AR(+/+) and (−/−) male mice at 6-month-old were provided in Fig. 2.

DISCUSSION

We have reported (16) that the pancreatic wet weight/body weight, and protein, chymotrypsin, and amylase contents in the pancreas were not different among CCK-AR(+/+), (+/−) and (−/−) female mice and that no histological differences were observed among the genotypes. In the present study, 14-day treatment with camostat significantly increased the ratio pancreatic wet weight/body weight compared with a control diet in CCK-AR(+/−) mice, although 3- and 7-day treatments did not reveal a significant effect.

When the effects of 14-day treatment with camostat were compared between CCK-AR(+/+) and (−/−) mice, the pancreatic wet weight, and protein and chymotrypsin contents in CCK-AR(+/+) mice were significantly higher than those in CCK-AR(−/−) mice. Amylase content tended to be lower in CCK-AR(+/+) than (−/−) mice, although the difference was not statistically significant, while amylase concentration (mg/g tissue) was significantly lower in CCK-AR(+/+) than (−/−) mice. Changes in enzyme compositions such as higher protease and lower amylase observed in CCK-AR(+/+) mice treated with camostat, were compatible with the previous results observed in rats, which were considered to be mediated via CCK and CCK-AR (2, 3, 7–12).

The initial body weight in CCK-AR(−/−) mice were significantly higher than those in CCK-AR(+/+). These 4 CCK-AR(−/−) mice in the present study could have been...
accidentally larger, because we and others (16, 21, 23) observed that the body weight in CCK-AR(−/−) mice were not different from other genotypes. We used progenies with the same birth date and raised altogether regardless of genotypes. When more than 2 rodents were maintained in one cage, the boss animal ate more and got larger.

Lacourse et al. (17) generated CCK-deficient mice and reported that 15-day treatment with a high protein diet significantly increased pancreatic protein content in both CCK(+/+) and (−/−) mice, and they concluded that pancreatic adaptation to dietary high protein did not require CCK (and CCK-AR). CCK and CCK-AR are not mandatory for the normal pancreatic growth in mice because the pancreas in CCK-AR(−/−) and (+/+) mice were not different, when raised by standard diets (16, 23), and adaptation to dietary high protein did not require CCK, either (17). However, oral administration of camostat requires CCK-AR to elicit the significant effect on the pancreas in mice. The body weight was significantly decreased by camostat treatment in both CCK-AR(+/+) and (−/−) mice. Therefore, feeding a diet containing camostat is not a physiological condition. The plasma volume obtained from mice was so small that we could not measure plasma CCK concentrations.

The trophic effects of oral administration of trypsin inhibitors could be observed within 7 days in rats (2, 3, 7–12). The present study, 14-day treatment with camostat was required to increase pancreatic wet weight and no increase was observed in the pancreas of CCK-AR(−/−) mice. Therefore, it is interpreted that camostat required CCK-AR to reveal a trophic effect on the pancreas in mice and that the pancreatic changes in CCK-AR(+/+) mice produced by camostat were obscure compared with rats.

As we did not measure DNA content in the present study, it is unknown whether the pancreatic hyperplasia was induced by camostat as reported in rats (2–12). The cell size examined by HE staining was not different between CCK-AR(+/+) and (−/−) mice after 14-day camostat treatment, but regardless of an increase in pancreatic wet weight, pancreatic hyperplasia could have been induced by camostat in CCK-AR(+/+) mice.

In conclusion, oral administration of camostat showed a trophic effect on the pancreas in mice, although the effect was less potent compared with rats. The trophic effect required CCK-AR in mice. Therefore, again, we do not anticipate the effect of camostat to enhance regeneration of the pancreas after pancreatitis besides to inactivate enzymes.

REFERENCES
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