Anti-obesity activity of hen egg anti-lipase immunoglobulin yolk, a novel pancreatic lipase inhibitor

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Abstract

Background: There is completely no report about both hen egg anti-lipase immunoglobulin yolk (IgY) and its anti-obesity action. Thus, we tried to isolate and characterize a novel anti-lipase immunoglobulin from hen egg yolk. Moreover, we investigated whether hen egg yolk anti-lipase IgY inhibits pancreatic lipase activity in vitro, and examined its ability to prevent obesity in a murine high fat diet-induced obesity model.

Methods: We determined the inhibitory action of Anti-lipase IgY on lipase activity in vitro. We also focused our evaluation on the anti-obesity properties of Anti-lipase IgY in a murine high fat diet-induced obesity model.

Results: Anti-lipase IgY blocked porcine lipase activity with an IC50 of 0.49 μM. Supplementing the high fat diet with only 0.2% (w/w) of Anti-lipase IgY for 35 days significantly decreased the weights of intraperitoneal adipose tissues, epididymal, mesenteric, retroperitoneal and perirenal adipose tissues, and the amounts of hepatic total lipid, triglyceride, and cholesterol. This was accompanied by a significant increase in the fecal excretion of triglyceride in the absence of diarrhea. Furthermore, Anti-lipase IgY treatment restored body weight gain to levels similar to mice fed with Control IgY.

Conclusions: This study provides the first report of the development of anti-lipase IgY and the direct evidence that inhibition of pancreatic lipase using Anti-lipase IgY is an effective anti-obesity treatment due to the associated increase in fecal excretion of triglyceride.

Keywords: Obesity, IgY, Pancreatic lipase, Lipid, Mice

Finding

Introduction

Obesity causes excess fat accumulation in several tissues in addition to white adipose tissue (WAT), such as other insulin-responsive organs including the skeletal muscle and liver; this predisposes individuals to the development of insulin resistance. Obesity is strongly associated with metabolic syndrome, which is characterized by the presence of insulin resistance, hypertension, and hyperlipidemia. Metabolic syndrome, which is closely linked to atherosclerosis, has become a major public health problem.

Several approaches for prevention and treatment of obesity have been reported [1]. Among these, both natural and synthetic pancreatic lipase inhibitors are effective in obesity prevention, likely due to their inhibition of intestinal lipid absorption. Indeed, a specific pancreatic inhibitor, Orlistat, has been used in the clinic for the prevention of obesity [2]. In animal studies, a major tea catechin, (−)-epigallocatechin-3-gallate [3] and polyphenol extracts in black tea [4] prevented high-fat diet-induced obesity via the inhibition of pancreatic lipase.

Another innovative candidate approach for pancreatic lipase inhibition is oral administration of anti-lipase immunoglobulin yolk (IgY) antibodies. There is completely no report about both hen egg yolk anti-lipase IgY and its anti-obesity action. Hens transfer immunoglobulin G (IgG) in blood into the egg yolk to provide acquired...
immunity to the offspring; this transferred IgG is termed IgY. The IgY technology offers several advantages over other methods of antibody production. For example, development and production of specific IgY can be achieved through collecting eggs from hens immunized with the target antigen, without the need for painful blood sampling and sacrifice of the animals.

The stability of IgY in pressure, heat, pH, trypsin, chymotrypsin, pepsin, and in the gastrointestinal tract has been well-documented [5]. IgY is relatively stable to pressure up to 4,000 kg per cm². IgY is stable at temperature ranging between 30°C and 70°C. It was found that the activity range of IgY for pH was pH 3.5 ~11. The stability of IgY at pH 3 was increased in the presence of sorbitol [6]. IgY is quite resistant against trypsin and chymotrypsin inactivation, but degraded by pepsin [7]. Moreover, our group has investigated the in vivo passage and the efficacy of IgY in the gastrointestinal tract of piglets and calves. Our results indicated that IgY powder was transported as immunologically functional molecules from the stomach down to the small intestine of calves while retaining much of their original biological activity [8].

Exploiting the peculiarities of the avian immune system, it is possible to mass-produce specific IgY antibody against various antigens, since IgY levels are extremely high in egg yolk, and one hen lays 250–300 eggs per year. Indeed, other scientists as well as we have produced IgY antibodies against microorganisms such as cholera [9] and Candida [10] using this method. Furthermore, we have shown that oral passive immunization with IgY antibodies prevented and improved antigen-induced disease symptoms in animal studies [11,12].

Here, we describe the production of a novel anti-lipase specific IgY from egg yolk following immunization of hens with a porcine pancreatic lipase, a key enzyme required for lipid digestion. Since the oral passive IgY-based immunotherapeutic strategy outlined above was effective against bacteria, we predicted that our new approach would produce a selective inhibitor of pancreatic lipase activity. By extension, we hypothesized that this inhibitor may exert anti-obesity effects. Thus, in this study, we investigated whether hen egg yolk anti-lipase IgY inhibits pancreatic lipase activity in vitro, and examined its ability to prevent obesity in a murine high fat diet-induced obesity model.

Methods and procedures
Reagents
The enzyme substrates and reagents were as follows: trio-lein, taurocholate, colipase, and L-α-phosphatidylcholine were from Sigma (MO, USA). High pure porcine pancreatic lipase was obtained from Elastin Products Co (Owensville, MO, USA). The fresh porcine pancreas was obtained from the local slaughtering house in Gifu, Japan. Lipase purification procedure was based upon the method of Garner and Smith [13]. Anti-porcine pancreas lipase-specific IgY containing preparation (designated as Anti-lipase IgY) and control IgY preparation (designated as Control IgY) were kindly provided by EW Nutrition Japan (Gifu, Japan).

Anti-Lipase IgY preparation
Five-month-old White leghorn hens (Hyline W36; Japan Layer, Gifu) were immunized according to the method described by Yokoyama et al. [14]. Briefly, the vaccine was prepared by mixing 0.5 mg of purified lipase antigen with 0.5 ml emulsion oil containing 5% Arlacel 80 (Maine Biological Laboratories, Waterville, Me, USA) and hens were immunized by injecting 0.5 ml to each of the breast muscles. Six weeks after the initial immunization, a booster was given in the same manner. Eggs from the immunized hens were harvested daily and stocked at 4°C. Egg yolk was separated carefully from the albumin. The yolk was then pooled, homogenized, and filtrated. Partially purified specific IgY powder was prepared by ammonium sulfate precipitate [15], and freeze-dry. Control IgY powder was prepared from the egg of non-immunized hens by the same method.

Assay to determine titer of anti-lipase specific IgY
The concentrations of both Anti-lipase IgY and Control IgY were determined using an enzyme-linked immuno-sorbent assay (ELISA) method as previously described [16].

Lipase activity assay
The porcine pancreatic lipase activity was determined using the method of Tsujita et al. [17].

Animals and diets
Male 6-week-old C57BL/6 J mice were purchased from Japan SLC (Hamamatsu, Japan). Mice were housed individually in standard plastic rodent cages, and placed in a room where the temperature was maintained at 22 ± 2.0°C with a 12-h light:dark cycle (lights on 0800–2000 h). All the mice consumed a commercial nonpurified MF (Mouse Flat) diet (Oriental, Yeast, Osaka, Japan) and tap water ad libitum for 4 days prior to their division into the following two weight-matched groups (n = 8; 8 mice per group): 0.2% (w/w) Control IgY-supplemented diet (CY) group, and 0.2% (w/w) Anti-lipase IgY-supplemented (AY) group. The compositions of the experimental diets are shown in Table 1. Mice were fed on these diets for 35 days. To determine the effective dose of Anti-lipase IgY in mice, we have done the preliminary experiment for 8 days. After this preliminary experiment, we have chosen 0.2% (w/w) of IgY in mouse study.
Food intake and body weights of the mice were recorded daily during the feeding period. At the end of the experimental period, animals were anesthetized with ether after a 22-h fasting period. Livers and visceral fat pads were collected and stored at −80°C until analysis. Fecal collections (d 7–9) were used for determining fecal lipids. The care and experimental procedures were approved by the Animal Care and Use Committee of Gifu University.

Biochemical analyses

Various lipid concentrations were determined using commercially available kits as follows: plasma, liver, and fecal triglyceride with Triglyceride E-test Wako (Wako Pure Chemical, Osaka, Japan) and plasma, liver, and fecal cholesterol with Cholesterol E-test Wako (Wako Pure Chemical, Osaka, Japan). Liver and fecal lipids were extracted by the method of Folch et al. [18], and total lipids were determined gravimetrically by the method of Nagaoka et al. [19].

Plasma glucose levels were determined by the glucose CII test (Wako Pure Chemical, Osaka, Japan). Plasma insulin levels were measured with an ELISA kit (Morinaga Institute of Biological Science, Yokohama, Japan). Plasma TNF-α levels were measured with an ELISA kit (R&D Systems, Minneapolis, USA).

Statistical analyses

All data presented in this study were normally distributed. Data were tested for normal distribution by the Kolmogorov-Smirnov normality test [20,21]. After Kolmogorov-Smirnov test, the statistical significance of differences was evaluated using the Student’s t-test [22].

Table 1 Composition of experimental diets

| Components       | CY  | AY  |
|------------------|-----|-----|
| Casein           | 286 | 286 |
| Control IgY      | 2   | -   |
| Anti-lipase IgY  | -   | 2   |
| Corn starch      | 172 | 172 |
| Sucrose          | 86  | 86  |
| Cellulose        | 65  | 65  |
| Soybean oil      | 40  | 40  |
| Lard             | 300 | 300 |
| Mineral¹         | 35  | 35  |
| Vitamin²         | 10  | 10  |
| Choline chloride | 4   | 4   |

¹AIN-93G Mineral mixture.
²AIN-93G vitamin mixture.

Results

Titer of anti-lipase specific IgY

The titers of Anti-lipase IgY (AY) or Control IgY (CY) used for the mouse study were 25.72 ± 1.37 mg/g or 0 mg/g preparation, respectively.

Inhibitory effect on lipase activity

AY inhibited pancreatic lipase activity by 95.4–18.3% at concentrations between 10,000–10 μg/ml, whereas CY did not inhibit triolein hydrolysis by pancreatic lipase. The IC50 value of AY was 88 μg/ml (0.49 μM). Growth inhibition of pancreatic lipase occurred in a dose-responsive manner (Figure 1).

Body weights, total food intake, fecal and tissue weights

Initial body weight, final body weight, body weight gains, total food intake, fecal and liver weights were all unaffected by dietary treatment (Table 2). The adipose tissue (epididymal, mesenteric, retroperitoneal and perirenal) weight was significantly lower in the AY group than that of the CY group.

Plasma parameters, liver and fecal lipids

The plasma triglyceride tended to be lower (p = 0.093) in the AY group than that of the CY group (Table 2).

Figure 1 Effects of increasing concentration of Control IgY (○) or Anti-lipase IgY (●) on pancreatic lipase activity. The values are means ± SEM, n = 6. Differences from the Control IgY were calculated using Student’s t test (*p < 0.05, **p < 0.01, ***p < 0.001).
we exploited a hen immunization model to produce
To begin the search for alternative anti-obesity agents,

Discussion

Liver triglyceride and cholesterol were significantly lower in the AY group than in the CY group. The fecal excretion of triglyceride was significantly higher in the AY-treated animals when compared with controls. Plasma glucose, insulin and TNF-α levels were not significantly changed between AY group and CY group.

Table 2 Effects of Control IgY (CY) and Anti-lipase IgY (AY) on physiological parameters

| Parameter                        | CY      | AY      |
|----------------------------------|---------|---------|
| Initial body weight (g)          | 27.57 ± 0.27 | 22.50 ± 0.38 |
| Final body weight (g)            | 26.18 ± 0.75 | 25.47 ± 1.01 |
| Body weight gain (g/35 d)        | 3.61 ± 0.88 | 2.97 ± 0.85 |
| Total food intake (g/35 d)       | 99.27 ± 4.81 | 95.28 ± 2.31 |
| Fecal dry weight (g/3d)          | 0.96 ± 0.03 | 0.91 ± 0.02 |
| Liver weight (mg/g B.W.)         |         |         |
| Liver weight                     | 33.6 ± 0.64 | 34.7 ± 1.07 |
| Epididymal WAT (g)               | 26.8 ± 2.76 | 20.2 ± 0.40* |
| Mesenteric WAT (g)               | 9.32 ± 0.94 | 6.57 ± 0.50* |
| Retroperitoneal WAT (g)          | 14.0 ± 1.18 | 10.8 ± 0.41* |
| Perirenal WAT (g)                | 9.51 ± 1.31 | 6.32 ± 0.40* |
| Intraperitoneal WAT (g)          | 59.6 ± 5.74 | 43.9 ± 0.73* |
| Plasma parameters                |         |         |
| Triglyceride (mg/dl)             | 54.2 ± 3.6  | 44.6 ± 3.9  |
| Cholesterol (mg/dl)              | 109.1 ± 3.9 | 104.6 ± 3.4 |
| Glucose (mg/dl)                  | 134.7 ± 4.1 | 137.1 ± 7.2 |
| Insulin (ng/ml)                  | 0.14 ± 0.02 | 0.14 ± 0.02 |
| TNF-α (pg/ml)                    | 3.29 ± 0.12 | 3.66 ± 0.17 |
| Liver lipids (mg/g liver)        |         |         |
| Total lipids                     | 132.6 ± 10.8 | 90.6 ± 7.9** |
| Triglyceride                     | 519.9 ± 0.9  | 450.0 ± 2.6* |
| Cholesterol                      | 6.77 ± 0.45  | 4.32 ± 0.17** |
| Fecal lipids (mg/3d)             |         |         |
| Total lipids                     | 71.4 ± 2.89 | 77.1 ± 2.48 |
| Triglyceride                     | 5.67 ± 0.32  | 7.50 ± 0.64* |
| Cholesterol                      | 6.65 ± 0.35  | 6.72 ± 0.26 |

1. Each value is the mean ± SEM, n = 8.
2. Asterisks indicate significant differences as compared with mice fed a high-fat diet containing control IgY by Student’s t-test (*p < 0.05; **p < 0.01).

To investigate the effect of anti-lipase IgY on the absorption of triglyceride in the diet, we analyzed fecal lipids contents. Previous study showed that lipase inhibition by synthetic lipase inhibitor Orlistat was extremely fast (half-inhibition time < 1 min) [25]. Moreover, in our preliminary study, single anti-lipase IgY administration tended to decrease in plasma triglyceride levels after single gavage of olive oil. These results indicate that the treatment with anti-lipase IgY for short term is effective for the increase in fecal triglyceride content. Thus, we performed fecal collection during day 7 through 9. On the other hand, it seemed to need longer time before the treatment with anti-lipase IgY became effective against the other measurements, such as adiposity and hepatic lipid accumulation. Thus, in this study, we investigated the effects of anti-lipase IgY treatment for longer period...
with the anti-lipase IgY on the high-fat diet-induced development. Obesity is defined as excessive fat accumulation [17]. Thus, we think we can expect that anti-lipase IgY has useful effect on body weight gain and obesity-induced metabolic disorders in the prolonged treatment.

Taken all together, the data from our study show that Anti-lipase IgY is a promising new lead biologic agent for the development of functional foods and medicines expected to prevent and improve obesity.

Competing interests
The authors declare that they have no competing interests.

Authors’ contributions
Contribution of each author: S N designed the research and wrote the manuscript; M H conducted the research and wrote the manuscript; T A, R S and K U conducted the research; Y K, S V, N, T G and M S analyzed the data. All authors read and approved the final manuscript.

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