STUDIES ON THE α-GLUCOSIDE HYDROLASE INHIBITOR, ADIPOSIN

IV. EFFECT OF ADIPOSIN ON INTESTINAL DIGESTION OF CARBOHYDRATES IN EXPERIMENTAL ANIMALS

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Adiposins showed a potent inhibitory activity against α-glucoside hydrolases such as salivary and pancreatic α-amylases, and intestinal disaccharidases *in vivo*. They suppressed the increase of the blood glucose level and the secretion of insulin in mice and rats which both had been fasted and then forced-fed on cooked corn starch, sucrose or maltose. The suppression of the increase in the body weight gains, and of the secretion of insulin and triglyceride in blood were observed in the experimental animals which were given diet containing adiposins. Hematological and histopathological examinations of animals treated with adiposins did not reveal any remarkable changes after a 3 months toxicity test. Adiposins did not show any intravenous acute toxicity in mice at dose levels (p.o.) less than 10 g/kg of body weight.

α-Glucoside hydrolases such as α-amylase and sucrase are known to be the key enzymes for carbohydrate digestion in the small intestine. Accordingly, an α-glucoside hydrolase inhibitor is expected to delay the hydrolysis of carbohydrates *in vivo* and it may be applicable to insulin-dependent diabetes, hyperlipemia and obesity as a supplementary agent of dietary cure.

As a result, adiposins, α-glucoside hydrolase inhibitors isolated from the cultured broth of *Streptomyces calvus* TM-521, were evaluated as worthwhile agents in insulin-dependent diabetes, hyperlipemia, and obesity.

The production, isolation and properties of adiposins* have been reported in the previous papers[1,2].

This paper deals with the result of the inhibition of carbohydrate digestion in the gastrointestinal tract and of the glucose absorption in the small intestine after the treatment of animals with adiposins.

Materials and Methods

Chemicals

Adiposin complex was partially purified by Amberlite IR-120 column chromatography and its chemical and biological properties were described in the previous paper[1,2]. Adiposin complex is expressed as adiposin in this paper.

Suppression Test of Blood Glucose Level in Mice

i) Animals and Feeding Condition: Male ICR strain mice (Charles River, Japan Inc.) with an average body weight of 20~22 g were fed the MF diet (Oriental Yeast Co., Ltd., Tokyo) for one week prior to drug administration. The mice were kept at 24±1°C and at 55±5% room humidity. The animals were kept in air-conditional rooms. Food was withheld from the mice 24 hours prior to each experiment. Free access to water was maintained.

* Adiposin-1 and -2 are identical to two of the amylase inhibitors described by OTANI *et al.*[8]

ii) Drug Administration: Adiposin was dissolved in physiological saline and administered orally by the stomach tube at doses of 132 GIU, 660 GIU and 3,300 GIU/mouse together with 1 g/kg of cooked corn starch, or with 2.5 g/kg of either sucrose or maltose. (One GIU is equivalent to decrease of 1 unit of glucoamylase under the conditions mentioned in previous paper[2].)

iii) Estimation of Blood Glucose: The blood glucose level was determined at short intervals after the administration of drug and carbohydrate by use of a Hitachi Model 500 Auto-Analyser (Tokyo).

Inhibition of Carbohydrate Digestion

i) Animals and Feeding Condition: Male Wistar strain rats (CLEA Japan Inc.) with the average body weight of 160~180 g were administered adiposin for the tests of the suppression of blood glucose, the secretion of serum insulin, and to know the inhibition of carbohydrate digestion in the gastrointestinal tract. Feeding and room conditions were similar to those described above. Food was withheld from the rats 48 hours prior to the experiment.

ii) Drug Administration: Adiposin was given at doses of 1,980 GIU, 6,600 GIU and 19,800 GIU/rat together with 1 g/kg of cooked corn starch.

iii) Estimations of Blood Glucose, Serum Insulin and Gastrointestinal Starch Content: Blood was obtained from rats by puncturing retroorbital venous plexus and the estimation of blood glucose was carried out using the Hitachi Auto-Analyzer. Serum insulin was estimated by the radio immunological assay (Insulin Kit, Dai-ichi Co., Ltd., Tokyo). For the estimation of starch in the gastrointestinal tract, rats were killed and the contents of stomach and small intestine were obtained by washing with cold water. The contents were hydrolyzed in 1 N sulfuric acid at 100°C for 3 hours and after neutralization of the hydrolysate, the free glucose was estimated by the phenol-sulfuric method.

LD_{50} Value of Adiposin

Adiposin was given to groups of animals, each consisting of ten male ICR mice, by the injection into the tail vein at a dose of 1, 5 and 10 g/kg each. The control group was given 0.2 ml of physiological saline. Seven days after the administration, the mice were observed for general symptoms.

Three Months Subacute Toxicity Test in Rats

i) Animals and Feeding Condition: Male SD strain rats (CLEA Japan Inc., Tokyo) with on average body weight of 215~260 g were used for the 3 months toxicity test. Feeding and room conditions were as mentioned above. Animals were divided into 4 groups, each group consisting of 8 rats.

ii) Drug Administration: Adiposin was mixed into CMF diet (Oriental Yeast Co., Ltd., Tokyo) and the rats were maintained on it for 3 months. Concentrations of adiposin in the diet were 36 GIU/g, 182 GIU/g and 908 GIU/g.

iii) General Observations: Food consumption and body weight were measured twice a week. Blood samples were collected once a month, before and after administration, from the retroorbital venous plexus; the hemoglobin concentration, hematocrit values were determined and the erythrocytes, platelets and leucocytes were counted. The following serum concentrations were determined: total protein content, albumin, A/G ratio, creatine, total bilirubin, GOT value, GPT value, free fatty acid, and Na^+ and K^+ levels, in addition, the animals were sacrificed and wet weights of heart, lung, liver, kidney, spleen, adrenal, testis and fat were recorded.

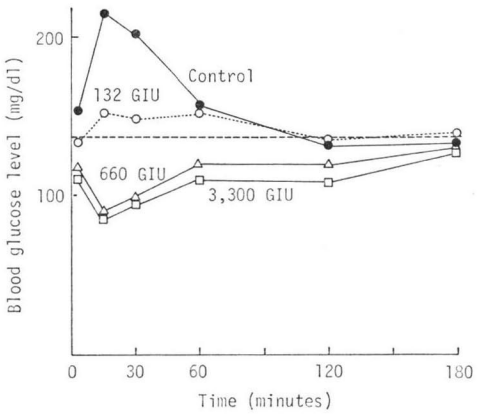
Results

Blood Glucose Level in Mice after Carbohydrate Loading with and without Adiposin

Within 15 minutes after the administration of cooked corn starch at 1 g per kg of body weight, blood glucose level rose from 150 to 220 mg/dl, as shown in Fig. 1. The administration of adiposin caused a dose dependent depression against this postprandial hyperglycemia. Ingestion of 2.5 g/kg

of sucrose or maltose by fasting mice resulted in peak blood glucose level at 15 minutes (285 mg/dl) and 5 minutes (355 mg/dl), respectively. The simultaneous administration of adiposin prevented this early postprandial rise of the blood glucose level significantly, as shown in Figs. 2 and 3.

Fig. 1. Effect of adiposin on blood glucose levels in fasting mice after oral administration of starch. (Administration: Cooked corn starch 1 g/kg)



Starch Content in the Gastrointestinal Tract of Rats

When rats were given 1 g/kg of cooked corn starch as an aqueous suspension, the starch was digested within two hours as shown in Fig. 4. Two hours after administration, only 12% of the starch was found in the gastrointestinal tract. On the other hand, when rats were given 1,980, 6,600 or 19,800 GIU/rat of adiposin admixed with cooked corn starch (1 g/kg), a significant amount of undigested starch was found in the gastrointestinal tract after 15, 30, 60 and 120 minutes, as compared with that found in the control animals which had been given cooked corn starch alone.

Blood Glucose and Serum Insulin in Rats after Starch Loading with and without Adiposin

As shown in Fig. 5, a loading of 1 g/kg cooked corn starch in rats resulted in an increase of serum insulin from 25 μU at 5 minutes to 52 μU/ml at 15 minutes. This rise was reduced by adiposin in a dose dependent manner.

Fig. 2. Effect of adiposin on blood glucose levels in fasting mice after oral administration of sucrose. (Administration: Sucrose 2.5 g/kg)

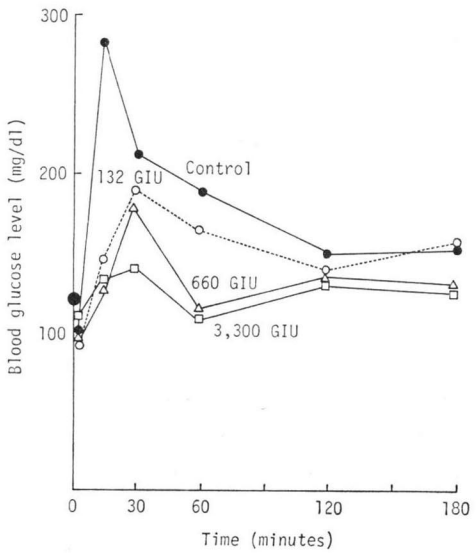


Fig. 3. Effect of adiposin on blood glucose levels in fasting mice after oral administration of maltose. (Administration: Maltose 2.5 g/kg)

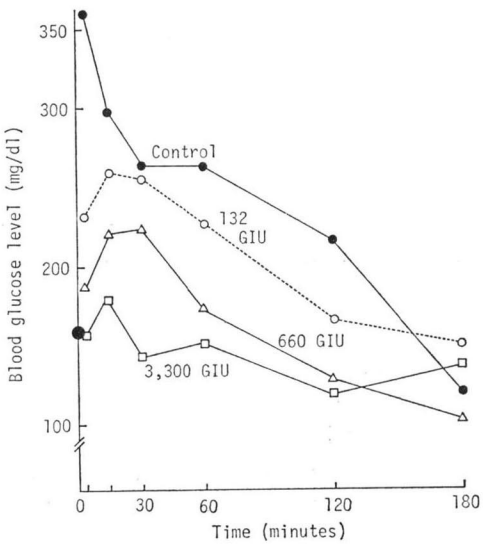


Fig. 4. Effect of adiposin on digestion of starch in fasting rats.
(Administration: Cooked corn starch 1 g/kg)

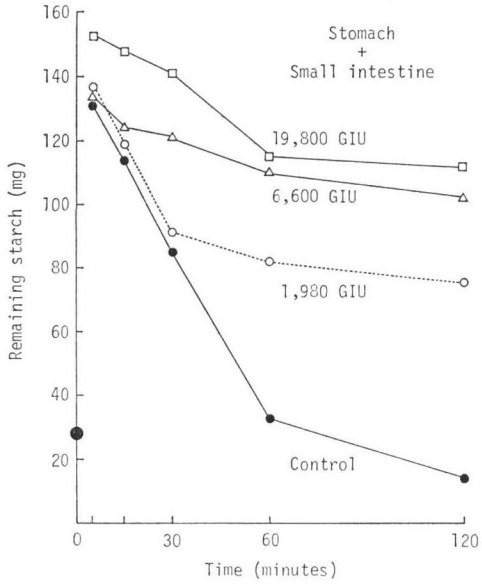


Fig. 5. Effect of adiposin on insulin and blood glucose in fasting rats after oral administration of starch.
(Administration: Cooked corn starch 1 g/kg)

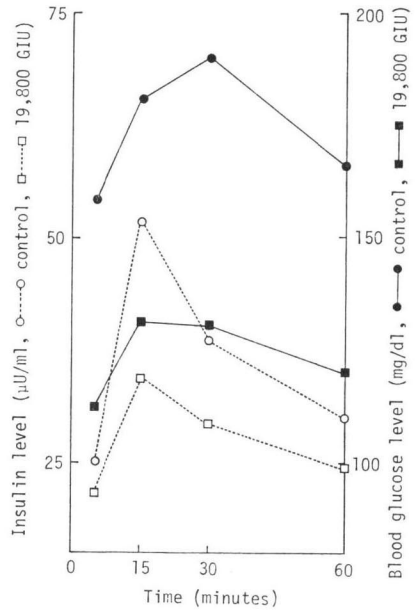


Fig. 6. Food intake of rats dosed orally with adiposin for 3 months.

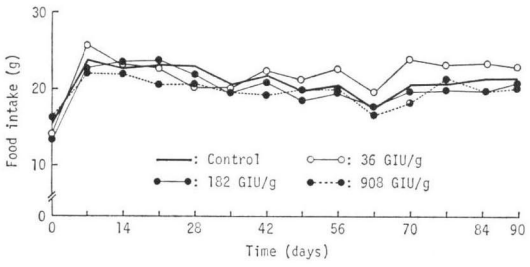
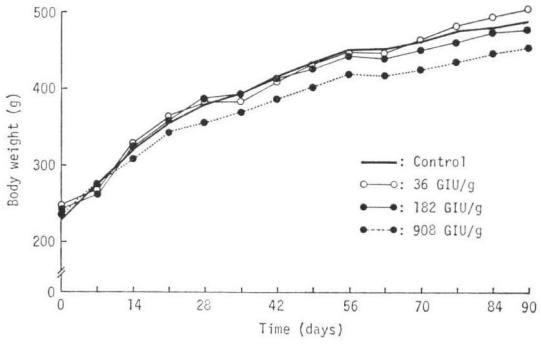


Fig. 7. Body weight changes of rats dosed orally with adiposin for 3 months.



LD_{50} Value of Adiposin

The LD_{50} value of adiposin in mice was estimated to be greater than 10 g/kg, since within the range of the doses of adiposin given, no animals died.

Rat Subacute Toxicity Test

Symptoms

Throughout the study, there was practically no reaction to the adiposin administration. In the group treated with the diet containing 908 GIU/g, a suppression of the body weight gain was observed from 10 days after the treatment. There was no significant change in the food intake among the groups treated with adiposin as compared with the control. No death was observed in any of the groups (Figs. 6 and 7).

Hematological Analysis, Serum Analysis and Organ Weights

No significant difference was observed in the hematological analysis, the serum analysis and in the organ weights between the treated and the control groups as shown in Tables 1, 2 and 3.

Macroscopic Observations at Autopsy.

No marked abnormalities were found between the treated and the control groups.

Table 1. Hematological findings in rats treated with adiposin.

		Control 9*	36 GIU/g 7	182 GIU/g 8	908 GIU/g 8
Red blood cell	($\times 10^4$/mm^3)	834±54	841±49	828±28	823±47
Hemoglobin	(g/dl)	16.2±0.3	16.1±0.5	16.2±0.2	16.0±0.7
Hematocrit	(%)	48.8±1.4	49.6±1.0	49.8±1.5	49.3±1.9
Blood platelet	($\times 10^8$/mm^3)	684±119	707±75	730±79	685±41
White blood cell	($\times 10^2$/mm^3)	65±11	66±10	70±13	70±5

* No. of animals

Table 2. Hematobiochemical findings in rats treated with adiposin.

		Control 9*	36 GIU/g 7	182 GIU/g 8	908 GIU/g 8
Total protein	(g/dl)	6.8±0.4	7.0±0.3	7.0±0.3	7.0±0.3
Albumin	(g/dl)	4.9±0.2	4.9±0.2	4.9±0.2	5.0±0.2
A/G ratio		2.6±0.3	2.5±0.2	2.5±0.2	2.6±0.2
Creatinin	(mg/dl)	2.4±0.2	2.5±0.3	2.5±0.4	2.5±0.6
Total bilirubin	(mg/dl)	0.0±0.0	0.0±0.1	0.0±0.0	0.0±0.0
GOT	(K-U)	124±21	135±26	134±28	136±36
GPT	(K-U)	68±20	86±42	78±39	81±40
Na	(mEq/l)	139±3	142±4	140±3	143±3
K	(mEq/l)	5.2±0.2	5.3±0.3	5.2±0.2	5.2±0.3
FFA	(mEq/l)	311±162	315±74	368±126	385±157

* No. of animals

Table 3. Organ weight per 100 g of body weight in rats treated with adiposin.

		Control 9*	36 GIU/g 7	182 GIU/g 8	908 GIU/g 8
Heart	(g%)	0.23±0.01	0.25±0.02	0.24±0.02	0.24±0.02
Lung	(g%)	0.30±0.03	0.29±0.03	0.30±0.03	0.28±0.04
Liver	(g%)	2.93±0.23	3.14±0.20	2.89±0.39	3.11±0.21
Kidney	(g%)	0.59±0.03	0.61±0.05	0.59±0.04	0.60±0.03
Spleen	(mg%)	141±16	140±9	142±9	145±18
Adrenal	(mg%)	10±1	12±1	11±1	11±1
Testis	(g%)	0.69±0.05	0.73±0.06	0.69±0.05	0.75±0.09
Fat	(g%)	4.58±0.58	5.00±0.85	4.68±0.99	4.75±1.00

* No. of animals

Discussion

It is well known that hyperglycemia sometimes occurs in animals and humans after the intake of

starchy foodstuffs or beverages due to the rapid digestion of starch by α-glucoside hydrolase in the gastrointestinal tract. This hyperglycemia is improved within the short time in healthy man and normal glucose levels are maintained; however in diabetics the hyperglycemia is particularly strong and long-lasting, and many disorders are observed by lowering of the carbohydrate metabolism function. Accordingly, we have studied the use of α-glucoside hydrolase inhibitors as therapeutic agents against carbohydrate-dependent metabolic diseases such as diabetes, hyperlipemia, hyperglycemia and obesity.

Recently, a number of these inhibitors have been isolated from the cultured broth of microorganisms by many researchers[3~11]. An α-glucoside hydrolase inhibitor, adiposin, isolated by the authors from the culture broth of *Streptomyces calvus* TM-521 exhibited inhibitory activities against α-amylase, sucrase and maltase *in vitro* as described in the previous papers[1,2]. When the fasting animals were given cooked corn starch together with adiposin, the postprandial increments of blood glucose and insulin were reduced significantly (Figs. 1 and 4). Similar results were achieved in loading tests using sucrose and maltose (Figs. 2 and 3). All these results indicate that adiposin suppresses the tentative increment of postprandial blood glucose and of the secretion of insulin by the inhibition of carbohydrate digestion.

Hence, adiposin may be a possible drug curative for diabetes where various disorders occur through the postprandial increment of insulin. The 3 months toxicity tests in rats, as shown in Tables 1, 2 and 3, reveal that there exists no significant difference between the group of adiposin treatment and that of the controls as judged from the results of hematological analysis, serum analysis, and macroscopic observations of organs. This demonstrates the safety of adiposin. As shown in Figs. 6 and 7, there were no remarkable changes in body weight gain or food intake in rats in the low dosage groups, but a slight inhibition of body weight gain was observed in the 182 GIU/g group treated with adiposin from eight weeks after the start of the treatment as compared with controls. In the 908 GIU/g group, weight suppression was observed from 10 days after the start. However, no significant changes in the food consumption were observed throughout the groups.

From these findings, adiposin is expected to serve as an interesting tool to induce a carbohydrate malabsorption through the inhibition of digestion and may possibly be of a beneficial therapeutic agent of dietary control in hyperlipemia, diabetes and obesity.

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