Inhibitory effects of aloe carboxypeptidase fraction on streptozotocin-induced enhancement of vascular permeability in the pancreatic islets.

Abstract

The protective actions of components isolated from Aloe arborescens Miller var. natalensis Berger (Kidachi aloe in Japanese) on streptozotocin (Sz)-induced necrosis of B cells in the pancreatic islets of the mouse were investigated to clarify its action mechanism involved in anti-diabetic effects. In this experiment, phenol low molecular weight components of aloin and aloin A that were anti-oxidants and derived from the leaf skin or pulp extract, an aloe carboxypeptidase fraction that is a inhibitor of enhanced vascular permeability and a glycoprotein component that decreases blood glucose were tested with mice precedently administered with Sz which is known as a cytotoxin specific to B cells. The results showed that the treatment group receiving Sz followed by the aloe carboxypeptidase fraction increased the inhibition of dye leakage by 75.8% ($p < 0.001$) in the extract of whole pancreas in comparison to the control group and the aloe carboxypeptidase fraction group also increased the inhibition effect by 68.4% ($p < 0.001$) in the extract of pancreatic islets as compared to the control group. The carboxypeptidase is an aloe-derived protease known to inhibit the acetic acid-related enhancement of intraperitoneal vascular permeability in mice. Further, the elevation of blood glucose in Sz-induced diabetic mice intraperitoneally given the aloe carboxypeptitase fraction was significantly ($p < 0.01$ - 0.001) restrained at 3, 7 and 14 days after the injection as compared to the control group given solvent only. The results of this experiment suggested that the inhibitory effect on the enhancement of vascular permeability related to the vascular acute inflammatory response at Sz-induced lesions of pancreatic islets was involved in the action mechanism of this enzyme.

Keywords: Aloe arborescens Miller; Carboxypeptidase; Anti-diabetes; Streptozotocin; Langerhans islets; Vascular permeability of the pancreatic islets

Introduction

There are plenty of articles related to the anti-diabetic effects of components that are of plant origin (Roman-Ramos et al., 1995; Alarcon-Aguilara et al., 1998; Reynolds and Dweck, 1999; Yeh et al., 2003; Eddouks et al., 2003). Polysaccharides like panaxan A, aconitan A and ganoderan B and C that exhibited hypoglycemic effects were found in Japan from galenicals such as Panax ginseng, Aconitum carmichaeli, Ganoderma lucidum

(Takahashi et al., 1985; Konno et al., 1985a, b; Tomoda et al., 1985, 1987, 1990; Hikino et al., 1985, 1989). These active polysaccharides were found to have a structure composed of glucan (Tomoda et al., 1984, 1986a, b), and their actions were reportedly to increase blood concentration of insulin and decrease glycogen in the liver of mice (Hikino et al., 1989).

On the other hand, there are several publications relevant to Aloe barbadensis Miller (Aloe vera Linne) and Kidachi aloe (Ghannam et al., 1986; Ajabnoor, 1990; Beppu et al., 1993; Chithra et al., 1998; Okyar et al., 2001), and Acemannan, glycoprotein and polysaccharides were cited as active ingredients. We have isolated hypoglycemic components from the mesophyll of Kidachi aloe that is a good source of polysaccharides (Beppu et al., 1990). Meanwhile, it was confirmed that in the leaf skin, there were some components exhibiting inhibitory actions of hyperglycemia as a result of the prevention of necrotic inflammation of pancreatic islet B cells (Beppu et al., 1990).

Streptozotocin (Sz) specifically destroys pancreatic islet B cells (Junod et al., 1967; Like et al., 1978; Agarwal, 1980; Cooperstein and Watkins, 1981). It was detected in the pancreatic islets of mice and rats that dye leakage related to increased vascular permeability reached a peak 4-8 h after Sz injection, when Monastral blue B or Evans blue was serially and intravenously injected (Sandler and Jansson, 1985; Beppu et al., 1987).

Majno et al. (1987) confirmed, using BB rats in Worcester colony (hereinafter referred to as "BB/Wor") of human insulin-dependent diabetes rat model, that the development of pancreatic vascular leakage (PVL) was intensified by the injection of Monastral blue B of a colloidal pigment into the tail vein of 20-200 days old BB/Wor rats. Nakano (1991) reported that PVL developed before insulitis manifested in BB/Wor rats. Majno and Doukas group found that intravenous injection of purified immunoglobulins from RT6-depleted diabetes-resistant (DR)-BB rat induced abnormal PVL in mice. The presence of immunoglobulins early in the disease process and their ability to induce PVL would suggest that they might participate in one of diabetes pathogenesis. More specifically, the pathomorphism of PVL would induce insulitis to be one of pathogenesis of diabetes mellitus (Doukas et al., 1996).

Now therefore, it is expected that the restraint of progressing PVL could retard a chain reaction of insulitis and the development of diabetes mellitus. The inhibition of vascular permeability in pancreatic islets may reduce the destruction of the pancreatic islets and retard the elevation of blood glucose, thus preventing the development of diabetes.

Previously, we pathologically found that a component of Kidachi aloe leaf skin showed more potent protective actions on B cell necrosis induced by Sz, which is known as a cytotoxin specific to B cells, than a component of Kidachi aloe leaf pulp.

We administered leaf skin and pulp extract components derived from a phenol low molecular weight component, a glycoprotein component and a carboxypeptidase fraction derived from Kidachi aloe to mice to compare the inhibitory effects on the Sz-induced enhancement of vascular permeability so that we can search any component participated in other action mechanisms to protect pancreatic islet B cells than the hypoglycemic action shown by glucans, Acemannan or other polysaccharides.

Materials and methods

Preparation of Kidachi aloe

Fresh aloe leaves (5-6 years old) were harvested from the herb garden of Yurika Co., Ltd. (Hisai, Japan). Leaves weighing over 80 g were selectively collected and processed immediately. Fig. 1 shows the diagram of the separation of aloe materials and yield quantities of samples used in the final experiment.

Preparation of freeze-dried whole leaf, freeze-dried leaf skin and freeze-dried leaf pulp of Kidachi aloe

Fresh whole Kidachi aloe leaves (3 kg) were homogenized in a polytron homogenizer and then freeze-dried (whole leaf FD). Using a knife, 3 kg of the fresh whole Kidachi aloe leaves were separated into the superficial layer of the leaf skin (leaf skin weighing approx. 1 kg) and the succulent layer of the leaf pulp (leaf pulp weighing approx. 2 kg), and prepared by the same method used for the preparation of the whole leaf freeze-dried powder. The leaf skin yielded a freeze-dried Kidachi aloe leaf skin powder (leaf skin FD), while the leaf pulp yielded a freeze-dried leaf pulp powder (leaf pulp FD).

Preparation of acetone-precipitated Kidachi aloe leaf skin juice

Leaf skins were removed from 3 kg of fresh whole Kidachi aloe leaves with a knife, homogenized in a polytron homogenizer and the homogenates were filtered through a Whatman GF/A filter. Approximately 1l (or 1 kg) of aloe leaf skin juice was treated with a two-fold volume of cold acetone, precipitated and lyophilized. The resulting powder was referred to as acetone-precipitated Kidachi aloe leaf skin powder (leaf skin AP).

[FIGURE 1 OMITTED]

Preparation of aloe carboxypeptidase from leaf skin AP powder by chromatography

Into 200 ml of 0.05 M acetate buffer (pH 5.0) including 0.2 M NaCl, 90 g of leaf skin AP powder was dissolved with a magnetic stirrer overnight, centrifuged at 18,000g for 15 min at 4 [degrees]C, and the supernatant was recovered. The supernatant was size-fractionated on a Sephadex G-25 ([phi]11.5 x 55 cm, Pharmacia Biotechnology, Uppsala, Sweden) with 0.05 M acetate buffer (pH 5.0) including 0.5 M NaCl.

The eluate was measured by absorption at 280 nm, then protein and monosaccharide concentration were measured and aloe carboxypeptidase (CPase) activity was determined. The eluate was divided among 50 test tubes (200 ml/tube). Each tube was divided into three fractionations (it was distributed between three peaks showing high sensitivity for an absorption wavelength of 280 nm), and freeze-dried to obtain CPase fraction in AP-F1 (leaf skin AP-F1).

Ammonium sulfate was added in the CPase fraction to yield up to 80% saturation. The precipitate was collected by centrifugation at 50,000g for 10 min, dissolved in 0.05 M acetate buffer with pH 5.0 containing 0.05 M NaCl and dialyzed against the same buffer.

The dialysate was applied to a column (11 x 15 cm) of DE-52 equilibrated with 0.05 M acetate buffer with pH 5.0 containing 0.05 M NaCl. The column was developed with stepwise gradients of NaCl of 0.15, 0.25, 0.4 and 1.0 M at a flow rate of 4 ml/min. Active fractions were collected, treated, with 80% saturation of ammonium sulfate and centrifuged at 50,000g for 10 min to precipitate salting out materials.

The precipitate was dissolved in 0.002 mM phosphate buffer with pH 6.5, dialyzed against the same buffer and applied to a column (3.3 x 20 cm) of Hydroxyapatite equilibrated with the same buffer. The column was eluted with stepwise gradients of phosphate of 0.002, 0.065, 0.1 and 0.5 M at flow rate of 2 ml/min. Then the eluant was freeze-dried to obtain the CPase fraction in AP-CPase (leaf skin AP-CPase fraction).

Preparation of 50 kD glycoprotein fraction from leaf pulp by ConA-Sepharose affinity chromatography

The previously described method (Beppu et al., 1990) was modified as follows; more specifically, 50 g of leaf pulp FD obtained by freeze-drying from the pulp of Kidachi aloe was dissolved into 900 ml of 0.05 M acetate buffer at pH 5.0. The solution was fractionated by a gel chromatography using Sephadex G-25 (113 x 550 mm) with the same buffer and a strongest hypoglycemic P1 fraction was obtained. The P1 fraction was substituted with 25 mM Tris-HCl buffer containing 0.5 M NaCl at pH 7.4, adsorbed to ConA-Sepharose (24 x 45 mm, confirmed by Pharmacia Biotechnology) and eluted with 0.5 M methyl-[alpha]-D-glycoside containing 0.5 M NaCl at pH 7.4. The eluate was fractionated by an affinity chromatography, and then the derived extraction fractions were further applied to a size-chromatography using Superdex-75 with phosphate-buffered saline (PBS) at pH 7.0 to yield a single fraction that was used for the experimentation.

Preparation of boiled Kidachi aloe leaf skin extract powder

The leaf skin was cut into small pieces, and put in a 31 glass beaker. To a wet weight of 200 g, 800 ml of distilled water was added, and then boiled for 40 min. The boiled extract was centrifuged at 18,000g for 15 min, and the supernatant was freeze-dried, yielding 2.4 g of powder (boiled leaf skin).

Chemicals

In this experiment, the main low molecular components of aloe, commercially available aloin (A-0451, barbaloin content: approx. 20%, Sigma Chemical Co., USA) and aloin A (B-6906, barbaloin content: approx. 97%, Sigma Chemical Co., USA), were used as control reagents. The components of aloe and barbaloin quantity (%) are shown in Fig. 1.

Measurement of dye leakage from the pancreas and pancreatic islets in an experiment of increased vascular permeability in the pancreatic islets of mice

Measurement of dye leakage from the pancreas and pancreatic islets was performed according to the method previously described (Beppu et al., 1987). Ten-week-old female ICR mice were divided into seven groups, and fasted for 15 h. In six of these groups, 160 mg/kg of Sz (Boehringer Mannheim, Germany) was intraperitoneally administered (Sz-

treated group). In the remaining group, only PBS was administered (untreated group). Furthermore, in five of the Sz-treated groups, 100 [micro]l/10 g body weight (powder dose: approximately 20 mg) of aloe components were intraperitoneally administered 30 min after Sz administration (Sz + aloe group). In the remaining group, PBS was administered (Sz + vehicle group).

Subsequently, in all groups, 50 [micro]l/10 g body weight of 2% Evans blue was intravenously injected 5.5 h after Sz administration. Thirty minutes later (6 h after Sz administration), the animals were exsanguinated via the heart under ether anesthesia. To extract the dye from the pancreatic islets, a laparotomy was performed under ether anesthesia, and the pancreas was removed to isolate the pancreatic islets.

The care and use of the animals were performed according to the "Guideline for the Care and Use of Laboratory Animals" of Fujita Health University.

Extraction of dye from the whole pancreas

Resected pancreatic specimens (n = 10 per group) were weighed in petri dishes on ice after the lymph nodes, fat tissues, and connective tissues were completely removed.

And then, the pancreas was placed in a heat-resistant glass test tube measuring 1.5 cm in diameter (Borosilicate glass round bottom), with a screw cap, mixed with 1.0 ml/0.1 g of pancreatic weight of Solvable[TM] (Packard, Holland), and heated with DRI-Block (DRI-Block DB-3H, M & S Instruments Trading Inc., Japan) at 80[degrees]C for 1 h to completely dissolve the pancreatic tissue. These solutions were allowed to return to room temperature, and then centrifuged at 7,700g for a few minutes. The supernatant was placed in a glass cell. Using water, the absorbance at 620 nm was measured, and dye concentrations were compared among groups.

Extraction of dye from the pancreatic islets

The previously described method (Beppu et al., 1987) was modified as follows; in mice (n = 5), a laparotomy was performed under ether anesthesia, and the duodenal side of the ductus choledochus was ligated, as described by Appels et al. (1989). Collagenase (Type IV, Funakoshi Co., Japan) was dissolved in Hanks' balanced salt solution (HBSS) to prepare a 1 mg/ml solution. Two milliliters of this solution was infused into the pancreatic duct, and the pancreas was removed.

The resected pancreatic specimens were heated at 37[degrees]C for 45 min to soften the whole tissue. After the extra-pancreatic islet tissues were removed, 100 pancreatic islets were collected under a stereoscopic microscope.

And then, HBSS-containing 100 pancreatic islets was placed in a heat-resistant glass test tube measuring 1.5 cm in diameter (Borosilicate glass round bottom) with a screw cap, and centrifuged at 900g for 5 min. After the supernatant was removed, 0.5 ml of Solvable [TM] (Packard, Holland) was added to the test tube, and heated with DRI-Block (DRI-Block DB-3H, M & S Instruments Trading Inc., Japan) at 80 [degrees]C for 30 min to isolate the pancreatic islets. The solution was placed at room temperature, and then

centrifuged at 7,700g for a few minutes. The supernatant was placed in a micro-glass cell (0.2 ml). Using water, O.D. was measured at 620 nm, and dye concentrations were compared among the groups.

Measurement of biochemical components

Hydrolysis levels were calculated using Z-Gly-Pro-Leu-Gly as a substrate, as described in our previous study (Ito et al., 1993) to measure the activity of CPase contained in Kidachi aloe. Hydrolysis levels of peptide and protein contained in leaf skin AP-CPase were also evaluated in the same manner using dipeptide, tetrapeptide, bradykinin, casein, [alpha]-N-benzayl-DL-arginine-p-nitroanilide (BApNA), N-acetyl-L-tyrosine ethyl ester (ATEE) and N-benzoyl-L-Tyr-ethyl-ester (BTEE). Also, the protease activity (Kunitz, 1947) and substrate specificity of the same serine protease group as aloe CPase were compared against pancreatic [alpha]-chymotrypsin of bovine origin (Type II Sigma, USA).

Monosaccharide levels were quantified by the phenol sulfuric acid reaction method (Hodge and Hofreiter, 1962). The protein levels were determined by the BCA protein assay reagent kit method (Pierce, USA).

Preparation of doses of aloe components for diabetes model animals and measurement of blood glucose

These Kidachi aloe-derived powders and chemicals were dissolved in PBS (pH 7.4) at concentrations of 1-200 mg/ml, centrifuged at 7,700g for 15 min, and filtrated with a 0.22 [micro]m filter. These solutions were intraperitoneally administered to mice.

Ten-week-old male ICR mice (five groups, n = 8) were fasted for 15 h with free access to water. According to the standard method (Like et al., 1978), 160 mg/kg of Sz was intraperitoneally administered to these mice. In four groups, leaf skin FD, leaf skin AP, boiled leaf skin and leaf skin AP-F1 were intraperitoneally administered as aloe components at a powder dose of 20 mg/100 [micro]l/10 g body weight 30 min after Sz administration. In the remaining group, mice were not treated after Sz administration.

To measure blood glucose, whole blood was collected from the retro-orbital vein plexus of mice that had been fasted for 3 h prior to blood collection, and 40 [micro]l of whole blood was quantified by glucose-HK test (Boehringer Mannheim, Germany) and glucose levels were calculated. Additionally, the same animal experimentation was performed using leaf skin AP-CPase obtained form hydroxyapatite gel chromatography. In this connection, after freeze-drying, leaf skin AP-CPase fraction was adjusted to a concentration at a powder dose of 20 mg/100 [micro]l/10 g body weight and administered to mice by intraperitoneal route.

Statistical analysis

All values are expressed as means [+ or -] standard deviation. Statistical significance was tested using Student's t-test for unpaired samples. p < 0.05 was regarded as significant.

Results

Inhibitory effects of Kidachi aloe components on increased vascular permeability in the pancreatic islets

As shown in Table 1, dye leakage suggesting increased vascular permeability in the whole pancreatic tissue and isolated islets was compared among the Sz + aloe groups, the Sz + vehicle group, and the vehicle group.

In the LE af skin FD group and the leaf skin AP group, absorbance (O.D.) values for the whole pancreas and isolated islets were significantly lower than those in the Sz + vehicle group. Among other groups, there were no significant differences. However, the leaf skin AP group showed more potent inhibitory effects than the leaf skin FD group. The boiled leaf skin did not show any inhibitory effects.

Light microscopic views of non Sz-induced or Sz-induced enhanced vascular permeability in the pancreas and isolated islets of ICR mice 30 min after intravenous injection of Evans blue are presented in Fig. 2. Photograph A shows the pancreas of a mouse given vehicle only. Photograph A shows dye leakage related to increased vascular permeability in the pancreatic islets 6 h after Sz administration. In the photograph, the pancreatic islets are observed as black spots. Sz-related acute vascular response (inflammation) increased vascular permeability in the pancreatic islets, resulting in dye leakage (black spots in Photograph) in the pancreatic islets. This finding was confirmed by black spots observed around the arrow. Likewise, Photograph C shows isolated pancreas islets of a Sz-untreated mouse and Photograph D shows isolated pancreatic islets of a Sz-treated mouse.

Gel chromatography of leaf skin AP and leaf skin AP-CPase fraction

The findings on the first Sephadex G-25 gel filtration chromatography of the leaf skin AP are shown in Fig. 3, and the absorption curve of the eluant at 280 nm, protein levels, monosaccharide, and CPase activity are described. Results of the final hydroxyapatite gel chromatography of DE-52 CPase fraction are shown in Fig. 4 in which absorption curve of the eluant at 280 nm and CPase activity are described. Active CPase fractions were collected (leaf skin AP-CPase fraction) and dialyzed against 0.005 M acetate buffer at pH 5.0 after lyophilizing. Table 2 shows the potential of enzymatic hydrolysis of leaf skin AP-CPase fraction. Assuming that the hydrolytic potential of leaf skin AP-CPase for Z-Gly-Pro-Leu-Gly is 100, it is shown to have a twofold hydrolytic potential against Z-Phy-Ty. However, the leaf skin AP-CPase did not react to Bz-Gly-Phe or Casein. On the other hand, it showed a high reactivity to Bradykinin. In addition, AP-CPase did not react to BApNA, ATEE or BTEE that are easily decomposed by pancreatic [alpha]-chymotrypsin of bovine origin.

[FIGURE 2 OMITTED]

Preparation of 50 kD glycoprotein fraction

Results of ConA-Sepharose affinity chromatographic analysis are presented in Fig. 5. And the molecular weight of purified material obtained through superdex-75 was estimated approximately 50,000. The compound was considered to be glycoprotein bearing [alpha]-

D-mannopyranose or [alpha]-D-glucopyranose on account of the specificity of the affinity chromatography.

[FIGURE 3 OMITTED]

[FIGURE 4 OMITTED]

Inhibitory effects of pharmacological components in aloe on increased vascular permeability in the pancreatic islets

As previously reported (Beppu et al., 1990), leaf skin AP-F1, leaf skin AP-CPase fraction and the 50 kD glycoprotein fraction, commercially available aloin or aloin A (barbaloin), which are the main low molecular weight components of aloe with high contents, were intraperitoneally administered to mice pretreated with Sz at a dose rate of 100 [micro]l/10g body weight (powder dose: approximately 20 mg). The results are shown in Table 3.

Leaf skin AP-CPase fraction and leaf skin AP-F1 potently inhibited dye leakage. However, neither 50 kD glycoprotein fraction nor commercially available aloin or aloin A (barbaloin) showed any inhibitory effects. The pancreas and blue-colored pancreatic islets of a mouse in the experimental group treated with Sz and vehicle are shown in Fig. 6A. Photograph B (Fig. 6B) shows the pancreas of a mouse in the experimental group treated with Sz and the leaf skin AP-CPase fraction. No pancreatic islet demonstrating black spots were observed.

[FIGURE 5 OMITTED]

Inhibitory effects of Kidachi aloe components on increased blood glucose in mice treated with a single dose of Sz

Boiled leaf skin, leaf skin FD, leaf skin AP and leaf skin AP-F1 were intraperitoneally given to mice 30 min after Sz administration. Changes in blood glucose are shown in Fig. 7. As a result, there was no deviation in the level of blood glucose in any group up to 12h after administration. In the leaf skin AP and leaf skin AP-F1 groups, the elevation in blood glucose was significantly inhibited and delayed when compared to that in the untreated group between 24 and 60 h after administration. However, in the leaf skin FD and boiled leaf skin groups, there were no inhibitory effects on the blood glucose level.

Additionally, in another similar study, leaf skin AP-F1 and leaf skin AP-CPase fraction were intraperitoneally administered to mice and observations were made for 14 days. Results of comparative blood glucose levels observed at 3, 7 and 14 days of the experiment are presented in Fig. 8. Leaf skin AP-F1 and leaf skin AP-CPase significantly reduced blood glucose levels between 3 and 14 days after administration in comparison with control treatment (vehicle alone).

Discussion

Majno et al. (1987) administered Monastral blue B pigment to BB/Wor rats of a spontaneous diabetes model, and reported that PVL was specifically enhanced in the

pancreas.

Furthermore, Nakano (1991) reported that Monastral blue particles were deposited on or passed through the basement membrane of the pancreatic venular endothelial cells in BB/Wor rats. In addition, he indicated that PVL occurred prior to the onset of pancreatic islet inflammation, was enhanced until the onset of diabetes, and then subsequently disappeared.

These findings suggest that the enhancement of PVL initially occurs in animals with spontaneous diabetes, and then insulitis develops, resulting in diabetes. It was reported that silica administration inhibited PVL and prevented diabetes (Oschilewski et al., 1985; Yanagawa, 1990; Nakano, 1991). Judging from the results obtained so far, it is considered that intraabdorminal macrophages, which are one of effectors for insulitis (Hanenberg et al., 1989; Yanagawa, 1990) are reduced in number due to the toxicity of silica particles to macrophages (Scott et al., 2002) to result in the restraint of their function of antigen-presenting ability or B cell impairment effect in pancreas islets (Hanenberg et al., 1989; Yanagawa, 1990; Nakano, 1991; Scott et al., 2002).

In our previous study (Beppu et al., 1987), the administration of a serotonin antagonist methysergide, which inhibits the enhancement of vascular permeability in pancreatic islets, inhibited blood glucose elevation. A subsequent experiment regarding the anti-diabetic effects of Kidachi aloe demonstrated that Kidachi aloe specifically protected pancreatic islets from Sz toxicity (Beppu et al., 1993).

In this experiment, when leakage of intravenously injected Evans blue was examined, leaf skin AP, which mainly consists of macromolecular components, more potently protected pancreatic islet cells than leaf skin FD but boiled leaf skin did not show any protective effects. Therefore, the protective effects may be associated with non-heat-resistant macromolecular components contained in leaf skin (Table 1). We detected CPase in Kidachi aloe leaves (Fujita et al., 1979), and reported that the CPase fraction inhibited the enhancement of vascular permeability related to burn injury induced at 56[degrees]C for 20 s (Fujita et al., 1980) as well as enhancement of intraperitoneal vascular permeability in an experimental inflammation model (Obata et al., 1993). To clarify the relationship between this enzyme and the protective effects on pancreatic islet cells, leaf skin AP was fractionated by Sephadex G-25 gel filtration chromatography and hydroxyapatite gel chromatography. The same fraction (leaf skin AP-F1, leaf skin AP-CPase fraction) as the CPase fraction showed the most potent protective effects (Table 3). This suggests that CPase is closely involved in the protective effects on pancreatic islet cells. In addition, we found that leaf skin AP-F1 inhibited the Sz-induced destruction of pancreatic islet cells, delaying blood glucose elevation (Fig. 5). This result was consistent with our previous finding (Beppu et al., 1993) that Kidachi aloe components relieved diabetic conditions in mice with Sz-induced diabetes.

[FIGURE 6 OMITTED]

Hikino et al. (1986) isolated polysaccharides (glycoprotein) of arborans A with a molecular weight of 12 kD and arborans B with a molecular weight of 57 kD as blood glucose-decreasing substances from whole Kidachi aloe leaves. They reported that in comparison

to that in vehicle-treated controls, the two substances significantly inhibited hyperglycemia in mice with alloxan diabetes for 7 and 24 h after administration, respectively. As reported by Hikino et al., we isolated a substance of 50 kD glycoprotein fraction with blood glucose-decreasing effects (Beppu et al., 1990). This substance markedly decreased blood glucose levels, as reported by Hikino et al., suggesting that it resembles arborans B (Beppu et al., 1993).

[FIGURE 7 OMITTED]

[FIGURE 8 OMITTED]

We performed a similar study using the 50 kD glycoprotein fraction that decreased blood glucose as reported by Hikino et al., However, the fraction did not inhibit Sz-induced enhancement of vascular permeability in pancreatic islets, as shown in Table 3.

Also a similar study was performed using aloe, which is a phenol component or an anthranoid glycoside with anti-oxidant and anti-inflammation actions (Yamamoto et al., 1991; Yagi et al., 2002; Shimpo et al., 2003; Beppu et al., 2003), contained in aloe and aloin A. As shown in Table 3, neither substance inhibited Sz-induced enhancement of vascular permeability in pancreatic islets.

Components that inhibit Sz-induced enhancement of vascular permeability in pancreatic islets, as investigated in this study, are substances that respond to the acinar portal vein of the pancreatic islets, but are not glycoproteins, such as arborans A or B, that directly decrease blood glucose levels (Table 3). In addition, the components may be present in the leaf skin, but not in the leaf pulp (Table 1). In this report, we found a component of Kidachi aloe leaf skin that prevented Sz-related injury of B cells in pancreatic islets, suggesting an association of this component with aloe CPase, a protease. Moreover, since the enzymatic nature of aloe CPase is different from that of other proteases of animal origin (Table 2), we wish to further investigate any specific affinity between pancreatic islets and aloe CPase.

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Table 1. Protective effects of aloe fractions on Sz-induced enhance vascular permeability in the pancreas of ICR mice 6 h after Sz administration--comparisons among whole leaf, leaf skin and leaf pu fractions

Treatment	Whole pancreatic tissue (n = 10)	
	Absorbance (b)	Inhibition (%) (c)
Sz + whole leaf FD	0.548 [+ or -] 0.085	15.1
Sz + leaf skin FD	0.502 [+ or -] 0.091*	35.1
Sz + leaf pulp FD	0.580 [+ or -] 0.156	1.3
Sz + boiled leaf skin	0.541 [+ or -] 0.098	18.2
Sz + leaf skin AP	0.482 [+ or -] 0.084**	43.7
Sz + vehicle(V)	0.583 [+ or -] 0.069	0
Vehicle	0.352 [+ or -] 0.038	--

Treatment	100 isolated islets (n = 5) (a)	
	Absorbance (b)	Inhibition(%) (c)
Sz + whole leaf FD	0.058 [+ or -] 0.022	26.7
Sz + leaf skin FD	0.045 [+ or -] 0.024*	44.0

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Sz + leaf pulp FD       0.077 [+ or -] 0.009              1.3
Sz + boiled leaf skin   0.075 [+ or -] 0.007              4.0
Sz + leaf skin AP       0.038 [+ or -] 0.018**           53.3
Sz + vehicle(V)         0.078 [+ or -] 0.008              0
Vehicle                 0.003 [+ or -] 0.001             --
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Statistical analyses by Student's t-test. Significant vs. control (vehicle): *p < 0.05, **p < 0.005.
(a) One hundred isolated islets/n.
(b) Values give absorbance units as mean [+ or -] S.D.
(c) Inhibition (%) = {[(Sz + V)-V]-[(Sz + aloe)-V]} x 100/[(Sz + V)-V].

Table 2. Substrate specificity of leaf skin AP-CPase and [alpha]-chymotrypsin

No. Substrate	AP Cpase Specific activity (Units/mg)	Relative activity (%)	[alpha]-Chymotrypsin Specific activity
1. Z-Gly-Pro-Leu-Gly (a)	1.22	100.0	0.0
2. Z-Phy-Tyr (a)	2.34	191.8	0.0
3. Z-Phe-Leu (a)	1.15	94.3	n.d.
4. Bz-Gly-Phe (a)	0.003	0.25	n.d.
5. Bz-Gly-Arg (a)	0.004	0.32	n.d.
6. Bradykinin (b)	6.24	511.5	0.0
7. Casein (c) (proteolytic activity)	0.00	--	0.6 A/hr/mg
8. BApNA (d) (hydrolytic activity)	0.00	--	0.8 A/hr/mg
9. ATEE (e) (esterolytic activity)	0.00	--	40 IU/mg
10. BTEE (f) (esterolytic activity)	0.0		25 IU/mg

n.d.: not detemined.
(a) One point 6 mM substrate was incubated with 0.25-2.0 [micro]g C or chymotrypsin at 37[degrees]C for 60 min. in 1.0 ml of 0.05 M ace buffer at pH 5.0.
(b) Arg-Pro-Pro-Gly-Phy-Ser-Pro-Phe-Arg.
(c) Twenty mM casein was incubated with 10 [micro]g CPase or Chymotrypsin at 37[degrees]C for 30 min in 900 [micro]g of 0.05 M b acid buffer at pH 7.4.
(d) [alpha]-N-benzayl-DL-arginine-p-nitroanilide.
(e) N-Acetyl-L-tyrosine ethyl estel.
(f) N-benzoyl-L-Tyr-ethyl-ester.

(d,e,f) Forty mM substrate was incubated with 50 [micro]g CPase or chymotrypsin at 25[degrees]C for 15 min in 600 [micro]l of 0.05 M Tris-HCl buffer at pH 7.8-8.0.

Table 3. Protective effects of aloe fractions on Sz-induced enhance vascular permeability in the pancreas of ICR mice 6 h after Sz administration--comparisons among Kidachi aloe components with diff sources and pharmacological actions

Treatment	Whole pancreatic tissue (n = 10)	
	Absorbance (2)	Inhibition (%) (3)
Sz + leaf skin AP-Fl (a)	0.475 [+ or -] 0.126	46.7*
Sz + leaf skin AP-CPase fraction (b)	0.408 [+ or -] 0.146	75.8**
Sz + 50 kD glycoprotein fraction (c)	0.576 [+ or -] 0.118	3.0
Sz + Aloin (d)	0.569 [+ or -] 0.092	6.1
Sz + Aloin A (d)	0.575 [+ or -] 0.085	3.5
Sz + vehicle (V)	0.583 [+ or -] 0.069	0
Vehicle	0.352 [+ or -] 0.038	--

Treatment	100 isolated islets (n = 5) (1)	
	Absorbance (2)	Inhibition (%) (3)
Sz + leaf skin AP-Fl (a)	0.042 [+ or -] 0.028	50.6*
Sz + leaf skin AP-CPase fraction (b)	0.028 [+ or -] 0.024*	68.4**
Sz + 50 kD glycoprotein fraction (c)	0.078 [+ or -] 0.056	5.1
Sz + Aloin (d)	0.069 [+ or -] 0.042	16.5
Sz + Aloin A (d)	0.075 [+ or -] 0.033	8.9
Sz + vehicle (V)	0.082 [+ or -] 0.012	0
Vehicle	0.003 [+ or -] 0.002	--

Statistical analyses by Student's t-test. Significant vs. control (
+ vehicle): *p < 0.05, **p < 0.001.
(1) One hundred isolated islets/n.
(2) Values give absorbance units as mean [+ or -] S.D.
(3) Inhibition (%) = {[(Sz + V)-V]-[(Sz + aloe)-V]} x 100[(Sz + V)-
Sample sources and their pharmacological actions:
(a) Crude fraction of aloe CPase by 1st chromatograph; serin protea
with anti-edema activity.
(b) Partial purification of aloe CPase by 3rd chromatograph; anti-
abdominal acetic acid inflammation and serin protease with anti-ede
activities.
(c) Purification from Kidachi aloe leaf pulp; hypoglycemic activity

(d) Phenolic compounds from Curacao aloe (Sigma A-0451); Radical scavenging and anti-inflammation activities.