Reduction of Blood Glucose Levels by Tea Catechin

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The influence of tea catechins on the absorption of starch or sucrose was investigated in vivo. Tea catechins were administered orally to rats before soluble starch or sucrose administration. Saccharide-dosed rats were killed and the blood and the contents of the intestine were collected at intervals over two hours. Catechins of certain concentrations suppressed the increase of plasma glucose levels, thus concurrently suppressing insulin activity. Increased activity of intestinal α-amylase by starch dosing was inhibited markedly in the catechin-administered rats. Sucrase on the brush border membrane was also inhibited by prior catechin administration. From these results it was assumed that orally administered catechins will inhibit intestinal α-amylase or sucrase, thereby deterring the digestion of certain amounts of starch or sucrose and eventually reducing the plasma glucose levels.

The interaction between plant polyphenols and proteins has been well known from early times, as is evident in for example, the practice of tanning hide to leather. However, the effects of plant polyphenols in the animal or human body, which are usually initially experienced as palatal astringency when consumed, have yet to be clarified. The presence of polyphenols in certain plant materials is known to decrease the nutritional value of forage crops such as sorghum or various fodder tree leaves. The reduced digestibility of these feeds may be explained, in part, on the basis of the inhibition of digestive enzymes. On the other hand, catechins in tea, although they are plant polyphenols, are confirmed to have a variety of physiological actions in preventing various causes of morbidity in rats. In this context, it is of great interest if tea polyphenols are found to interact with digestive enzymes in vivo to affect metabolic systems.

We have previously reported the inhibition of α-amylase by tea polyphenols in vitro. Tea catechins have further been proved to inhibit the activities of sucrase and α-glucosidase which were crudely scraped off the mucosal brush border of rat’s small intestine.

In this study, the influence of tea catechins on the absorption of starch or sucrose was investigated in rats. Tea catechin was administered orally before starch or sucrose administration, and the plasma glucose levels, plasma insulin levels as well as intestinal enzyme activities were examined over time.

Materials and Methods

Tea catechin. A tea catechin fraction, having 90% total catechin purity, was extracted from green tea as “crude catechins” (referred to as “catechin” hereafter).

Animals. Male Wistar strain rats weighing 180 to 200 g (6 weeks of age) were fed a commercial diet (Oriental Co., Ltd.) for a week. The room temperature was controlled at 23 ± 2°C and lighting was on a 12 h cycle (0700–1900: light conditions).

The rats were divided into 4 groups of 20 and starved overnight. Catechin solutions of 80, 60, and 40 mg/ml, were administered orally in 1 ml doses to each group of rats. Water was administered to the control group. After 30 min, 4 ml of 40% soluble starch solution was administered orally to all the rats. Immediately after administration, and at 30 min, 1 h, and 2 h intervals thereafter, rats from all groups were killed and their blood was collected. The same procedure was followed for sucrose, with catechin solutions of 80, 10, and 5 mg/ml being administered to the test groups. The plasma was stored at −20°C until the measurement of glucose and insulin levels. The contents of the small intestine of the rats administered starch and the brush border membrane of the rats administered sucrose were collected. α-Amylase activity in the intestine and sucrase activity on the brush border membrane were measured.

Measurement of blood glucose and insulin. Concentrations of glucose and insulin in the blood were measured using the glucose oxidase kit and the insulin-EIA test (Wako Pure Chemical Ind., Osaka), respectively.

Preparation of small intestinal mucosa. The mucosa was scraped off the small intestine with a slide glass and homogenized with 10 mm potassium phosphate buffer (pH 7.0). The homogenate was used for the assay of sucrase activity. Protein in the homogenate was measured by the ninhydrin method.

α-Amylase assay. α-Amylase activity in the intestine was measured by a modification of the method of Willstätter–Schudel. The contents of the small intestine were filtered and the filtrate was used for the assay of α-amylase. One ml of the filtrate was added to 1.0 ml of 0.1 M phosphate buffer (pH 6.9) and 2.0 ml of 4% soluble starch. The reaction mixture was incubated for 5 min at 37°C. The reaction was stopped by adding 3.0 ml of 0.1 N I₂-KI solution and 5.0 ml of 0.1 N NaOH. After 15 min, 1.0 ml of 2 N H₂SO₄ was added to the mixture and titrated with 0.02 N sodium thioulsate, using starch as an indicator. One unit of α-amylase activity was defined as the amount of enzyme which liberated 1 μmol of maltose per min.

Sucrase assay. The small intestinal mucosa homogenate was diluted in a ratio of 1:4 with 0.2 M maleate buffer (pH 6.0), before use in the experiment. The reaction was started by adding 100 μl of 400 mm sucrose solution to 100 μl of the sample and the mixture was kept at 37°C for 10 min. The reaction was stopped by adding 200 μl of DNS reagent and put into a boiling water bath for 5 min. After the addition of 2.0 ml of distilled water, the absorbance was measured at 540 nm by the Shimadzu model UV-220 spectrophotometer. One unit of sucrase activity was defined as the amount of enzyme which liberated 1 mmol of glucose per min.

Statistical analysis. Statistical evaluation was done with Student’s t-test. Differences were considered to be statistically significant when p < 0.05.

Results

When 80 or 60 mg of catechin were given 30 min before the administration of starch, the increase of glucose and insulin concentrations in the plasma was significantly suppressed as compared with those of the control group (Fig. 1). However, when 40 mg of catechin was given before...
starch administration, there was only slight suppression, confirming that suppression was dependent on the quantity of catechin administered to the rats. In the case of sucrose, 80 mg of catechin significantly suppressed the rise of glucose and insulin levels in the plasma which would otherwise have been elevated by sucrose (Fig. 2). Similar effects were observed with 10 mg of catechin administration, but no effect was observed with 5 mg of catechin.

The intestinal α-amylase activity of the catechin group (80, 60, and 40 mg) scarcely increased during the 2 h after the administration of starch, but the enzyme activity of the control group increased markedly after the starch dosing (Fig. 3). The sucrase activity in the catechin groups (80, 10, and 5 mg) was significantly lower than with that of the control group (Fig. 4).

**Discussion**

α-Amylase in saliva or in pancreatic juice, and sucrase, maltase, isomaltase, lactase, and trehalase on the brush border membrane are all enzymes related to the digestion of carbohydrates. We have previously proved in vitro the inhibitory activity of tea polyphenols on α-amylase or on intestinal disaccharidases. These findings prompted us to examine how these in vitro activities of tea polyphenols would be realized in actual animal bodies. Various amounts of catechins were administered to rats followed by a full, but tolerable amount of starch 30 min afterwards. At intervals over two hours after saccharide administration, plasma glucose and insulin concentrations, as well as intestinal enzyme activities were measured. The results showed the inhibition of α-amylase in the intestine, which was presumably responsible for the resultant suppression of plasma glucose and plasma insulin levels. There appears
to be a certain threshold concentration for catechin to suppress the plasma glucose levels. Simultaneous administration of starch with catechin had similar results (data not shown). Sucrase activity and plasma glucose levels were affected in the same way by prior or simultaneous administration of catechin with sucrose.

The difference between the inhibition patterns of α-amylase and sucrose further implies the interaction of these enzymes with catechin. α-Amylase is secreted when starch enters the duodenum. Therefore, there was hardly any difference between the inhibition of control and catechin-fed groups immediately after starch administration (0 min). At 30 min, when starch was present in the duodenum, enzyme activity peaked and was markedly inhibited by various doses of catechin. On the other hand, sucrose is always present on the brush border membrane of the small intestine. Results show a definite inhibition of sucrose at 0 min, suggesting that the prior catechin dosing worked to suppress sucrase regardless of sucrose administration.

Plasma insulin concentration increased proportionate to that of plasma glucose, and both were duly suppressed by catechin. However, in the case of sucrose administration, plasma insulin fluctuation patterns did not correspond to those of plasma glucose. In this case, it might have been that the plasma glucose level peaked a little earlier than 30 min (not analyzed) and the suppression of insulin was manifested a little later, as shown in the graph.

Throughout these experiments, it was postulated that the inhibition of enzymes by tea polyphenols plays a key role in the suppression of plasma glucose levels. Accelerated insulin secretion or the deterrence of glucose absorption into the body, are other possible factors that will suppress the increase in plasma glucose levels. However, it was confirmed in previous experiments that catechin has no influence on these factors (data not shown). That is, the administration of catechin alone did not affect the pattern of insulin activity. In the same way, when glucose was given, catechin administration brought about no change in the pattern of plasma glucose level variations.

It could be concluded from these experimental results, that the inhibition of the above saccharidases by catechin was realized in the small intestine of rats and as a result, when a surfeit of starch or sucrose was administered, an increase in glucose levels was suppressed. In another series of experiments, it was confirmed that the amount of feces on a dry weight basis increased nearly twice as much as those of the control in rats fed catechins over a few days.11,12 This increase was found to gradually level off over a longer period of catechin feeding, implying that there is a certain degree of indigestibility of catechins and supplemental secretion of α-amylase.

It was also confirmed that as much as 1 or 2% catechin in the diet, fed over a period of three months, did not reduce body weight gains or food intakes as compared with those of control rats.13 These results imply that the possible indigestibility of catechin would not cause any malnutrition.

These experiments studied the effects of single administration of catechin, starch, or sucrose. Detailed feeding experiments over a longer period are necessary to examine the influence of catechins on the absorption and excretion of carbohydrates in rodents as well as in humans. Various such experiments are presently under way.

References