〈Original Article〉

Difference of inhibitory effect of α-glucosidase by tealeaves species and extraction condition and effect of black tea on postprandial blood glucose level elevation in ICR mice

Naoko Uchida[1)], Nao Yoshimoto[2)], Asae Nakamura[3)], Ayumi Takagi[4)], Kayoko Honda[4)], Mihoko Moto[1)], Kaname Katsuraya[1)] and Naotaka Hashizume[3)]

Summary Inhibitory effects in vitro of α-glucosidase within various kinds of black tea leaves were evaluated. Comparison of 24 different black tea leaves showed that the Uva is the most effective black tea for α-glucosidase inhibition. The Uva tea was extracted in various conditions and their inhibitory effect was compared. The highest activity was obtained in the brewing condition of 4% tea leaves against hot water (80℃), for 3 min. The test on ICR mice revealed that the black tea has significantly inhibitory effect to the elevation of Postprandial blood glucose (PBG) levels caused by the loading of starch, maltose and sucrose. As a result of measurement of total polyphenol quantity of 24 kinds of black tea, positive correlation was shown between total polyphenol quantity and inhibitory effect on α-glucosidase activity of the black tea ($p < 0.001$). Finally, relationship between chemical component and activity was examined by using of HPLC method. These results indicate that the inhibitory effect of the black tea on α-glucosidase activity is influenced by the difference in tea varieties, and the extraction condition. In addition, it was suggested that the black tea suppressed the elevation of PBG levels, and theaflavin was the main active ingredient of inhibitory effect on α-glucosidase activity among polyphenols of the black tea.

Key words: Black tea, α-glucosidase, Blood glucose, Theaflavin, HPLC method

1. Introduction

Black tea, oolong tea, and green tea are derived from the tea leaves of the same species (*Camellia cinensis*), but different manufacturing processes result in completely different tastes and flavors of each tea.

Black tea is classified as fermented tea, oolong tea as semi-fermented, and green tea as non-fermented, based on the differences in the degree of fermentation during manufacturing. 80% of the tea production worldwide is black tea, and its history dates back to more than 400 years[1)]. Among black tea, Darjeeling, Uva, and Keemun are the world's three major tea varieties. In Japan, Assam is widely available in

[1)]Faculty of Human Ecology, Wayo Women's University Graduate School
2-3-1 Kounodai, Ichikawa, Chiba 272-8533, Japan
[2)]Graduate School of Pharmaceutical Sciences, Chiba University
[3)]Department of Health and Nutrition, University of Human Arts and Sciences
[4)]Department of Health and Nutrition, Wayo Women's University

Received for Publication January 8, 2013
Accepted for Publication January 16, 2013

addition to these three varieties. The polyphenols in tea have been reported to have inhibitory effects on hyperglycemia[2] and enhance insulin sensitivity[3] in addition to having antioxidative[4], anti-allergic effects[5], and promoting antidiabetic effects. According to a publication by the International Diabetes Federation (IDF), the number of individuals with impaired glucose tolerance (IGT) in the world as of 2007 was 308 million, and this number was estimated to increase to 418 million by 2025. Correcting postprandial hyperglycemia has been demonstrated to be clinically effective in preventing the development and progression of diabetes[6]. This led to an approach to suppress the digestion and absorption of carbohydrates by inhibiting the activity of carbohydrate digestive enzymes such as α-amylase and α-glucosidase to mitigate the elevation of postprandial blood glucose (PBG) concentrations[7-12]. Inhibitory effects of the extracts of Guava[7], *Ginkgo biloba* L.[8], *Acanthopanax*[9], and Mulberry[10] leaves on α-glucosidase activity has been previously reported, and their inhibitory effects on the elevation of PBG levels have been investigated in animal and human studies. However, the inhibitory effect of black tea on elevated PBG levels in vivo has been quite insufficient. Moreover, it is also insufficient consideration of the components involved.

This paper confirms, to start with, the inhibitory effect of black tea on α-glucosidase activity *in vitro*. Thence the difference of inhibitory effect of α-glucosidase by tealeaves species and extraction condition is investigated. In addition, carbohydrate tolerance tests with male ICR mice are performed to investigate the influence of black tea on the elevation of PBG levels when loaded with soluble starch, maltose, or sucrose. Finally, the relationship between α-glucosidase inhibitory activity and theaflavins as a polyphenols is investigated by measuring with HPLC method.

2. Materials and Methods

1. Preparation of enzymes

Maltase and sucrase solutions were prepared according to the method described by Deguchi et al.[7] Ninefold rat small intestinal acetone powder (w/v; 1630-10G, SIGMA-ALDRICH Co., St. Louis, USA) was added to 56 mM maleic acid buffer (pH 6.0) and homogenized in ice using a homogenizer (AS ONE Co., Japan). The homogenate was centrifuged (3000 rpm for 10 min at 4℃), and the supernatant was used as a crude enzyme solution. The crude enzyme solution was diluted 20- and 2-fold with 56 mM maleic acid buffer (pH 6.0) to evaluate maltase and sucrase activity, respectively.

2. Inhibitory effect of the twenty four types of black tea on α-glucosidase activity

From the 24types of commercially available black tea varieties [Darjeeling (India), Uva (Sri Lanka), Assam (India), and Keemun (China) from 6 companies], black tea extracts were prepared by 3 g of each type of leaf with 200 mL of hot water for 3 min according to the method described by Tanaka et al.[13] and used after filtration (No. 2, 110 mm).

An aliquot (0.5 mL) of 2% maltose solution was added to an equal volume of each black tea sample and then 0.5 mL of maltase solution was added. The mixture was then incubated at 37℃ for 90 min. For control, 56 mM maleic acid buffer (pH6.0) was added as instead of black tea. After incubation, the reaction was terminated by heat inactivation of the enzyme in a boiling water bath for 10 min. In blank, the enzyme was heat inactivated in a boiling water bath for 10 min, immediately after the addition of the enzyme without to incubate at 37℃. After terminating the reaction, the amount of glucose in the supernatant was determined using the Glucose CII-test Wako kit (Wako Pure Chemical Industries, Osaka, Japan). The rate of enzyme inhibition was calculated from the glucose content using the following formula:

Rate of enzyme inhibition (%) = {(produced glucose content in control − produced glucose content when added with black tea)/produced glucose content in control)} × 100

Similarly, sucrase activity was calculated using 2% sucrose solution and sucrase solution. Tests were repeated six times to obtain the average and the standard error value.

3. Influence of the extraction temperature of black tea on the total theaflavins content

Black tea samples were prepared by filtration (No. 2, 110 mm) 100 mL of water containing 1.5g of Uva leaves after extraction at cold water (3℃), water at room temperature (19℃) and hot water (80℃) for 30 min.

The control standard was using tea extract (SIGMA-ALDRICH Co., St. Louis, USA). This standard product was a mixture of four types of theaflavins (theaflavin, theaflavin-3-monogallate, theaflavin-3'-monogallate and theaflavin-3, 3'-digallate), and theaflavins purity were about 80%. Calibration curve was created based on the assumption that the purity of theaflavins is 80%. The amounts of total theaflavins were calculated based on the calibration curve.

The HPLC system consisted of the TOSOH CO-8010, CCPS, SD-8022 and the UV-8020 detector set at 375 nm (Tosoh Co., Japan). Data analysis was performed using the Chromato-PRO (ver. 3.0) data analysis software (Run Time Co., Japan). TSKgel ODS-80Ts column (4.6 mm ϕ, 250 mm, Tosoh Co., Japan), and was operated at 40℃. The mobile phase adjusted consists of water, acetonitrile and phosphoric acid (76:23:1). The flow rate was 1.0 ml/min.

4. Influence of the amount of black tea leaves on the total theaflavins content

Black tea samples were prepared by filtration (No. 2, 110 mm) 100 mL of hot water containing 0.5, 1.0, 2.0, 4.0, and 5.0 g of Uva leaves after extraction at 80℃ for 3 min. The amounts of total theaflavins were measured by HPLC in the same method described above.

5. Influence of the extraction time of black tea on the total theaflavins content

Black tea samples were prepared by filtration (No. 2, 110 mm) 100 mL of hot water containing 4.0 g of Uva leaves after extracting for 10 s, 1, 3, and 30 min. In the 30 min extraction, the water temperature was maintained using a refrigerator and a hot plate. The amount of total theaflavins was measured by HPLC in the same method described above.

6. Animals and rearing conditions

Male ICR mice (Clea Japan Inc., Tokyo, Japan) weighing 35-40 g were used in the experiments. They were reared under a 12 h light-12 h dark cycle maintained in a room at 23 ± 1℃ and 55 ± 5% humidity and fed the solid pellets of CE-2 (Clea Japan Inc., Tokyo, Japan). Mice were provided with food and water *ad libitum*. All experiments were conducted in accordance with the laboratory animals' welfare guidelines of the Standards Relating to the Care and Management of Laboratory Animals. In addition, this study has been approved by the Ethics Committee of Wayo Women's University on biological and epidemiologic research using animals in accordance with the Declaration of Helsinki.

7. Carbohydrate tolerance test on mice using various carbohydrates

Black tea samples were prepared by filtration (No. 2, 110 mm) 100 mL of hot water containing 4.0 g of Uva leaves after extraction for 3 min. Three types of carbohydrates (soluble starch, maltose, and sucrose) were used for loading. After 18 h of fasting, the mice were assigned separately to control and black tea groups (n = 5) so that the average fasting plasma glucose level of each group was equalized. According to the method described by Deguchi et al.,[7] the control and black tea groups were orally administered with 0.6 mL of water or black tea, respectively, and after 30 min, carbohydrates ware loaded (2 g/kg body weight) performed in each group. Before and 15, 30, 60, and 120 min after carbohydrate loading, blood samples were collected from the tail veins of the mice and the blood glucose levels were determined using the Glutest Ace R compact blood glucose meter (Sanwa Kagaku Kenkyusho, Aichi, Japan).

8. Measurement of the total polyphenol in the twenty four types of black tea

The total polyphenol amount of 24 types of black tea was quantified by the colorimetric analysis based on the ferrous tartrate method[14]. Ferrous tartrate reagent was prepared by dissolving 100 mg of iron (Ⅱ)

sulfate heptahydrate and 500 mg of potassium sodium tartarate in distilled water, and adjusted the total volume to be 100 ml.

50 μl ferrous tartrate and 150 μl phosphate buffer (1/15M) were mixed with 50 μl of each tea, and the optical density was measured at 540 nm.

Catechins of the black tea can be classified in four types which are (+) catechin, (-) epicatechin, (-) epigallocatechin, and (-) eppigallocatechin gallate. In the present study, the most frequently used (+) catechin was selected as the standard reagent, and the total amount of polyphenol was measured by the composed optical density.

9. Measurement of the theaflavins in the 24 types of black tea by HPLC

The total polyphenol amounts of 24 types of black tea were measured by HPLC in the same method described above. The control standard was using tea extract (SIGMA-ALDRICH Co., St. Louis, USA). This standard product was a mixture of 4 types of theaflavins (theaflavin, theaflavin-3-monogallate, theaflavin-3'-monogallate and theaflavin-3,3'-digallate). Therefore, calculate the sum of the 4 peak areas.

The amount of theaflavin was also measured by HPLC in the same method described above. The control standard was using theaflavin (Wako Pure Chemical Industries, Ltd., Japan). The amount of theaflavin was calculated from the peak areas.

10. Statistical analysis

Values are mean ± standard error. IBM SPSS statistics (Ver. 20; IBM, New York, USA) was used to test significance. Tukey's test and Student's *t*-test were used to investigate the inhibitory effect of black tea on α-glucosidase activity and changes in the elevation of blood glucose levels after carbohydrate loading, respectively.

In addition, Pearson's correlation coefficient was used to investigate the correlation between polyphenols and inhibitory effect of α-glucosidase activity. P-value < 0.05 was considered statistically significant. Regression analysis tool of Excel 2010 (Microsoft) was used for multiple regression analysis.

3. Results

1. Inhibitory effects of the 24 types of black tea on α-glucosidase activity

Figure 1 shows the inhibition rates of the 24 types of black tea (Darjeeling, Uva, Assam, and Keemun, each tea purchased from 6 companies A to F) against maltase and sucrase activity. These data ware line up with inhibition rates, 4 companies out of 6 exhibited Uva having the strongest activity, followed by Darjeeling, Assam and Keemun. Amongst of them all, Uva from company A showed highest activity while Keemun was the lowest in all 6 companies. Similar tendency was observed in sucrose activity, and Uva of the company A showed the highest inhibition.

2. Influence of the extraction temperature of black tea on the total theaflavins contents

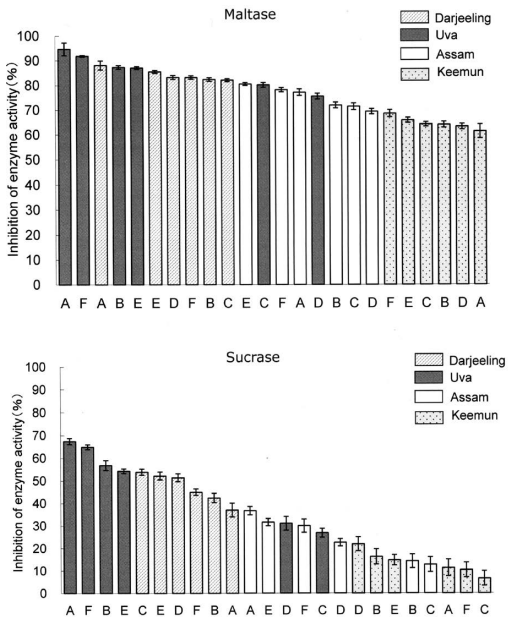

Fig. 1 Effect of 24 kinds of black tea leaf extracted solution on the maltase or sucrase activity. Darjeeling, Uva, Assam and Keemun, in total of 24 samples from 6 companies (A~F), were used to compare inhibitory effects on maltase and sucrase in vitro. Inhibitory effect was defined as compared with control reaction at 90 min in which glucose was 100%. Mean ± SE (n=6).

Figure 2 shows the total theaflavins contents when Uva was extracted with cold water (3℃), water at room temperature (19℃), and hot water (80℃) for 30 min. The total theaflavins concentration was the highest 147.3 ng/mL when extracted with hot water. The total theaflavins concentration was 64.4 ng/mL when extracted with water at room temperature, 15.6 ng/mL when extracted with cold water. The data suggests that the total theaflavins content is correlated with its extraction temperature.

3. Influence of the amount of black tea leaves on the total theaflavins contents

Figure 3 shows the total theaflavins contents, using the amount of tea leaves of 0.5%, 1.0%, 2.0%, 4.0%, and 5.0% (w/v) in 100 mL of hot water for 3 min. The total theaflavins contents of black tea were 29.6 ng/mL, 142.2 ng/mL, 264.6 ng/mL, 610.1 ng/mL using 0.5, 1.0, 2.0, 4.0 w/w% tea leaves respectively. The total theaflavins contents increased proportionally with the concentration of tea leaves up to 4.0 w/w%. However, the total theaflavins contents of 5.0 w/w% black tea was 29.6 ng/mL, it was same degree amount of the total theaflavins contents of 4.0 w/w% black tea.

4. Influence of the extraction time of black tea on the total theaflavins contents

Figure 4 shows the total theaflavins contents, using concentrations extracting 4.0 g of tea leaves for 10 s, 1, 3, and 30 min in 100 mL of hot water. The total theaflavins contents of black tea were 36.6 ng/mL, 60.0 ng/mL, 92.8 ng/mL extracting time was for 10 sec, 1 min, 3 min respectively. The total theaflavins contents increases proportionally with the extraction time of tea leaves up to 3 min. However, the case of tea leaves extracting for 30 min, the total theaflavins content was 84.8 ng/mL, it was same degree amount of the total theaflavins content of tea leaves extracting for 3 min.

It was, therefore, concluded that the inhibitory component against α-glucosidase activity was most efficiently extracted when 4 g of tea leaves were used in 100 mL of hot water for 3 min. Such extracts were used in the subsequent experiments.

5. Influence of the administration of black tea on blood glucose concentrations after carbohydrate administration

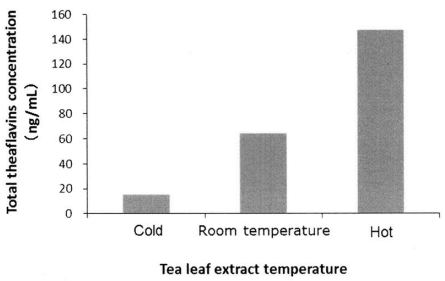

Fig. 2 Effect of black tea leaf extracted solution to cold, room temperature or hot water on total theaflavins contents. Black tea was extracted to cold, room temperature or hot water at 30min. Total theaflavins were measured by the HPLC method.

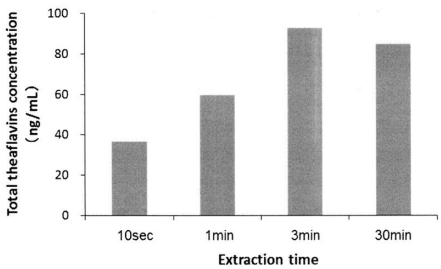

Fig. 3 Effect of black tea leaf extraction time on total theaflavins contents. Black tea was extracted to hot water at 10sec, 1, 3, or 30min. Total theaflavins were measured by the HPLC method.

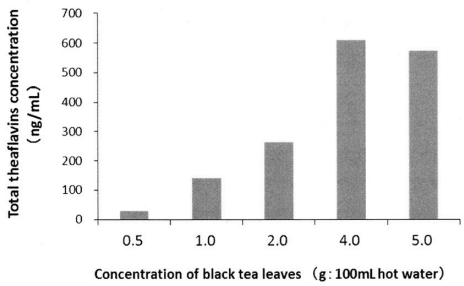

Fig. 4 Effect of black tea leaf concentration on total theaflavins contents. Black tea was extracted to 100mL hot water at 0.5, 1.0, 2.0, 4.0 or 5.0 g. Total theaflavins were measured by the HPLC method.

Figure 5 shows the changes in blood glucose concentrations in mice which were orally administered 0.6 mL of black tea extract followed by the loading of soluble starch, maltose, or sucrose at 30 min later. In the case of starch loading, the blood glucose concentrations at 30 min later was 281.4 ± 18.6 mg/dL in the control, while in the black tea group, the figure was 226.6 ± 16.3 mg/dL, indicating a significantly lower value ($p < 0.05$). When maltose was administered, the blood glucose concentration of the black tea tended to be lower than control at 30 min later to maltose administration. And the blood glucose concentration of black tea administration (112.4 ± 12.2 mg/dL) was significantly lower than control (175.5 ± 21.9 mg/dL) at 120 min ($p < 0.05$). For sucrose administration, the blood glucose concentration of black tea administration (130.6 ± 9.8 mg/dL) was significantly lower than control (174.8 ± 17.5 mg/dL) at 15 min ($p < 0.05$). And the blood glucose concentration of black tea administration (145.8 ± 11.0 mg/dL) indicated significantly lower than control (224.2 ± 29.1 mg/dL) at 30 min ($p < 0.05$).

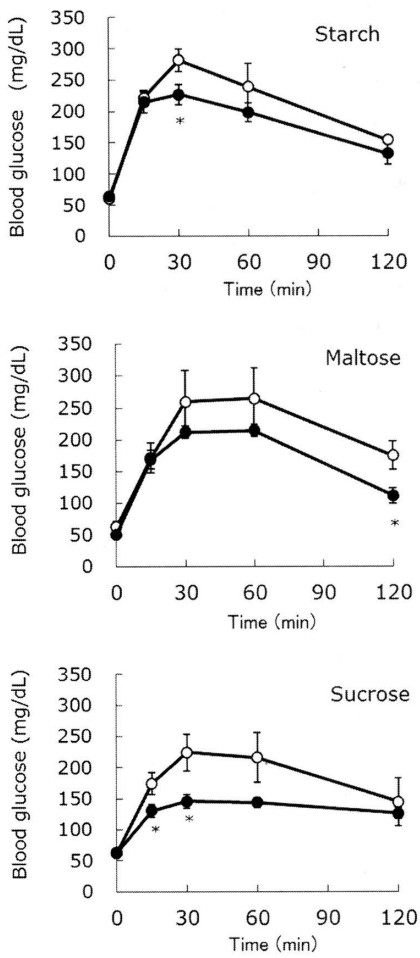

Fig. 5 Effect of black tea on the PBG level in maltose or sucrose or starch loaded mice. After being fasted for 18 h, mice were loaded sugar (2g/kg body weight) at 30 min after administration of black tea or water. Blood glucose was measured at 0, 15, 30, 60, 120 min after the loading carbohydrates. Mean±SE. ○, control with water ; ●, black tea administrated. (n=5). *p＜0.05 vs control with water.

6. Total polyphenol content

Figure 6 shows the relationship between the total polyphenol, as measured by the ferrous tartrate

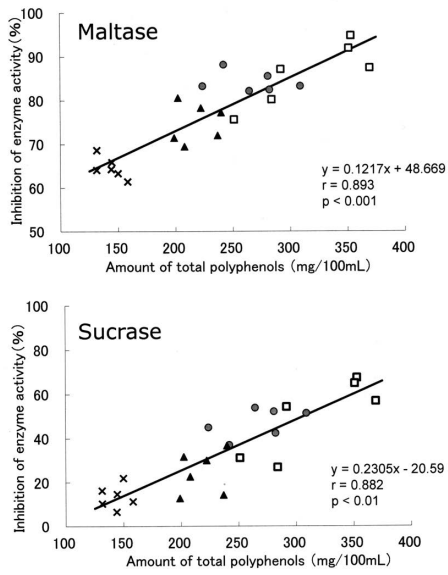

Fig. 6 The relationship between inhibition of enzyme activity and the amount of total polyphenols of twenty four kinds of black tea. Inhibitory effect was defined as compared with control reaction at 90 min in which glucose was 100%. □, Uva ; ●, Darjeeling; ▲, Assam; ×, Keemun. Darjeeling, Uva, Assam and Keemun (n=24).

Table 1 HPLC peak area and concentration of theaflavins in twenty four kinds of black tea[a)]

Theaflavins	Peak area	nmol/L[b)]
Non-substituted theaflavin	53030 ± 6930	124.5 ± 16.3
Theaflavin - 3 - monogallate	37240 ± 4450	n. d.
Theaflavin - 3'- monogallate	19520 ± 2550	n. d.
Theaflavin - 3, 3'- digallate	35290 ± 4700	74.6 ± 9.9
Total	145080 ± 16440	n. d.

[a)] 24 kinds of black tea ware measured theaflavins peak area by HPLC method.
[b)] Concentration was obtained from a calibration curve which was made by using pure compounds. Mean ± SE (n=24).

method, and maltase and sucrase activities. Total polyphenol showed a meaningful positive correlation between maltase activity ($r = 0.893$; $p < 0.001$) and sucrase activity ($r = 0.882$; $p < 0.001$). Based on these results, Uva was concluded as the most promising black tea in terms of inhibiting the α-glucosidase activity and was used in all of the subsequent experiments.

7. Study of component with action to inhibit the α-glucosidase activity

In order to calculate the peak area of 4 types of theaflavin from 24 different kinds of black tea samples were measured by HPLC method, and presented the results in Table 1. The results showed that theaflavin has the highest content of component with action to inhibit the α-glucosidase activity with the mean value of 53030±6930 followed by theaflavin-3-monogallate with 37240±4450, theaflavin-3,3'-digallate with 35290±4700, and theaflavin-3'-monogallate with 19520±2550 in this order. Moreover, correlation between peak area of 4 types of theaflavin and active inhibition ratio of maltase and sucrase were examined. The results showed that the coefficient of correlation with theaflavin was the highest, and there was a significant positive correlation confirmed between maltase active inhibition ratio ($p<0.001$) and sucrase inhibitory effect ($p<0.01$). However, there were no significant correlations between the area of theaflavin-3-monogallate, theaflavin-3'-monogallate, theaflavin-3,3'-digallate and the active inhibition ratio of maltase and sucrase.

Meanwhile, the concentration of theaflavin for 24 different kinds of black tea were measured, and the results are presented in Figure 7. The results of this

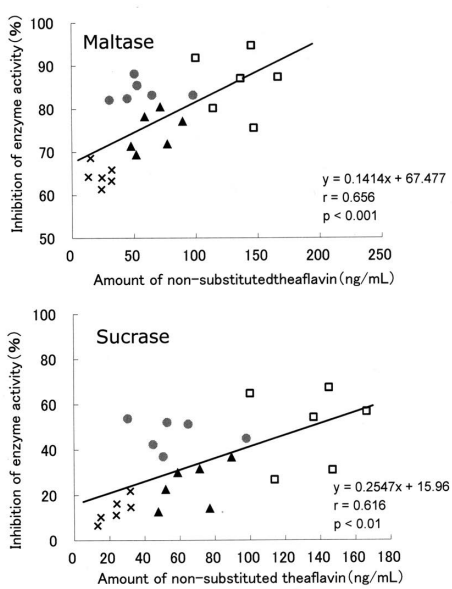

Fig. 7 The relationship between inhibition of enzyme activity and the amount of non-substituted theaflavin of twenty four kinds of black tea. Inhibitory effect was defined as compared with control reaction at 90 min in which glucose was 100%. □, Uva; ●, Darjeeling; ▲, Assam; ×, Keemun. Darjeeling, Uva, Assam and Keemun. (n=24)

investigation showed that there is a significant positive correlation between the concentration of theaflavin and inhibitory effect ratio of maltase ($r=0.656$; $p<0.001$) or sucrase ($r=0.616$; $p<0.01$).

4. Discussion

In vitro comparison of the 24 types of black tea (Darjeeling, Uva, Assam, Keemun from 6 companies respectively) revealed that the inhibitory effect on α-glucosidase activity was the highest in Uva. Therefore, it is suggested that the difference in the inhibitory effect on α-glucosidase activity is related to the difference in the manufacturing environment etc., in addition to the difference of the kind of tea leaves. By this suggestion, it was the most reasonable selection to use the Uva (which is manufactured by Company A) for the following experiments using the highest inhibitory effect on α-glucosidase activity

Extraction condition of black tea was examined by using theaflavins as an indicator, which is known as a characteristic polyphenol of black tea. There are 4 existing kinds of theaflavin: theaflavin, theaflavin-3-monogallate, theaflavin-3'-monogallate, and theaflavin-3,3'-digallate. LC/MS method, which is a combination of HPLC method and Mass Spectrometry method (MS), is a well-used measurement method of polyphenols nowadays; however, HPLC method can only be used when the reference material of the target is already available in the market. Then, peak areas of 4 kinds of theaflavin were calculated by HPLC method and a comparative investigation was conducted by calculating the total amount of theaflavin from the total peak area. First, optimal extraction temperature was verified by measuring total theaflavin amount after extracting for 30 min by maintaining temperature at different conditions, such as cold water (3℃), water at room temperature (19℃), and hot water (80℃). By the results of this experiment, it was found that the higher temperature, provide higher concentration of the total theaflavin in black tea.

Secondly, an optimal condition for extraction concentration and time were examined. The results of this examination showed that the total theaflavins concentration depend on both of the extraction concentration and time. However, there were no difference observed at conditions with concentration more than 4.0 w/w% and with time longer than 3 minutes. Tanaka et al.[13] have reported that there was no difference observed in the inhibitory effect on α-amylase activity at extraction time in 3 minutes and 2 hours. Therefore, it was judged that the component involved in the inhibitory effect on α-glucosidase activity could be extracted sufficiently at the condition of 4.0 w/w% of tea leaf concentration and 3 minutes as a extraction time. The black teas extracted in these conditions were used for our later experiments.

Carbohydrate tolerance tests in mice using starch, maltose, and sucrose for carbohydrate loading were performed. The results show the tendency of the PBG concentrations to decrease in black tea-administered groups compared with those in the control group, irrespective of the carbohydrate loaded. Starch, maltose, and sucrose comprise the majority of carbohydrates ingested daily by humans. Black tea has an inhibitory effect on α-glucosidase, indicating it is potentially effective in inhibiting the elevation of PBG concentrations. In the present study, monosaccharide was not used because of the focus on the inhibitory effect on α-glucosidase to evaluate the inhibitory effect of black tea on the elevation of PBG concentrations; however, our glucose administration test using rats has confirmed that the administration of black tea tended to inhibit the elevation of blood glucose concentrations (unpublished data). This indicates that other action mechanisms, apart from the significant inhibitory effect on α-glucosidase activity, are possibly involved in the inhibitory effect of black tea on the postprandial elevation of blood glucose. Nishiumi et al.[15] reported that green and black tea promote glucose uptake by muscle cells. In addition to α-glucosidase inhibition, other mechanisms of the inhibitory effect of black tea on the elevation of PBG levels need to be investigated.

In this study, our results indicated that the theaflavin is the most effective component of black tea for the α-glucosidase inhibition. However, there is a no clear relationship was observed between other theaflavines such as, theaflavin-3-monogallate, theaflavin-3'-monogallate, theaflavin-3,3'-digallate and the α-glucosidase inhibition of black tea. This result indicated that, theaflavin can be identified as the main component of the inhibitory effect on α-glucosidase activity of black tea. Hara et al.[16] have reported that theaflavin-3,3'-digallate showed the highest inhibitory effect against α-amylase activity among 4 kinds of theaflavin. Moreover, Honda et al.[17] has

reported that theaflavin-3,3'-digallate showed the highest inhibitory effect on α-glucosidase activity among theaflavins. According to these reports, it was expected that the theaflavin-3,3'-digallate have a highest activity of inhibition of α-glucosidase. However, in accordance with the results of this study, it was found that the inhibitory effect was controlled by the theaflavin in the fact, because of the high proportion of its existence.The reason for this can be that the content of theaflavins is the main involvement for the inhibitory effect from this study.

From the results of HPLC measurement of 24 kinds of black tea, it was found that the concentration of non-substituted theaflavin was significantly higher than substituted theaflavins. While it was repoted that the theaflavin-3,3'-digallate was the most effective inhibiter against the α-glucosidase, theaflavin-3,3'-digallate has not become the most effective component. It was estimated to due to the concentration of theaflavin-3,3'-digallate was only half of the concentration of non-substituted theaflabin. As a result of multiple regression analysis between the inhibitory effect and the content of theaflavins by HPLC analysis, it has been found that an inhibitory effect against α-glucosidase of theaflavin-3,3'-digallate is 1.4 times larger than non-substituted theaflavin. As a result of the multiple regression analysis are described together without contradiction the fact of our experiments in this study and report of Honda et al.[17] Because non-substituted theaflavin is contained significantly larger amount than theaflavin-3,3'-digallate, α-glucosidase inhibitory effect is exclusively dominated by the concentration of non-substituted theaflavins. In addition, multiple regression analysis for theaflavin-3-monogallate and theaflavin-3'-monogallate indicated that these two components does not contribute to α-glucosidase inhibition. From these results, not only the strength of the inhibitory effect against α-glucosidase activity of a component but also the content of the component in whole food is considered to be important for the investigation of effective ingredient. As a result, it was indicated that the non-substituted theaflavin is the main component having the inhibitory effect against the α-glucosidase activity in black tea.

5. Conclusion

These results indicate that the inhibitory effect on α-glucosidase activity of black tea is influenced by the difference in tea varieties. Tea components showed the rise with the increase in the concentration of the tea leaves and the extraction time. However, the results showed that the amount of the components extraction become nearly flat, more than that the tea leaves extracting 4 g against 100 mL hot water, for 3 min. In addition, an inhibiting trend of elevation of PBG levels of black tea was observed in all carbohydrates from starch, maltose, and sucrose tolerance test in mouse. The results indicate that black tea is useful for suppression the elevation of PBG levels. Moreover, from the results of the investigation of a participating component, a strong association with polyphenols was observed, which indicates that theaflavin is the main component involved in the inhibitory effect on α-glucosidase activity in black tea. The functional mechanism of the inhibitory effect on α-glucosidase activity and an impact from long-term ingestion of black tea on the human body are chosen for further study.

Acknowledgments

The authors would like to thank H. Umemoto and K. Kano for their technical skills and helpful discussions and H. Kato from Smooth Link Inc. for his useful comments.

References

1) Sano M, Saito Y: Health function and culture of tea. Vol1, 58-75, Ai Kei Corporation Inc., Tokyo (2008)
2) Litao Z, Julie KF, Michael DL: An extract of black, green, and mulberry teas causes malabsorption of carbohydrate but not of triacylglycerol in healthy volunteers. Am J Clin Nutr, 84: 551-555, 2006.
3) Heping C, Isabelle HF, Meghan AK, Rachida B, Harry DD, Sara Coves, Anne MR, Richard AA: Green tea polyphenol extract regulates the expression of genes involved in glucose uptake and insulin signaling in rats fed a high fructose diet. J Agric Food Chem, 55: 6372-6378, 2007.
4) Anton R, Sheila W: Antioxidant Effects of Tea: Evidence from Human Clinical Trials. J Nutr, 10: 3285-

3292, 2003.

5) Maeda-Yamamoto M, Ema K, Shibuichi I: In vitro and in vivo anti-allergic effects of 'benifuuki' green tea containing O-methylated catechin and ginger extract enhancement. Cytotechnology, 55: 135-142, 2007.

6) Bischoff H: Pharmacology of alpha-glucosidase inhibition. Eur J Clin Invest, 24 (3): 3-10, 1994.

7) Deguchi Y, Osada K, Uchida K, Kimura H, Yoshikawa M, Kudo T, Yasui H, Watanuki M: Effects of extract of guava leaves on the development of diabetes in the db/db mouse and on the postprandial bood glucose of human subjects [Jpn]. Nippon Nogeikagaku Kaishi, 72: 923-931, 1998.

8) Pinto Mda S, Kwon YI, Apostolidis E, Lajolo FM, Genovese MI, Shetty K: Potential of Ginkgo biloba L. leaves in the management of hyperglycemia and hypertension using in vitro models. Bioresour Technol, 100(24): 6599-6609, 2009.

9) Moto M, Tamura A, Yamada N: Inhibitory effects of himeukogi leaf tea on postprandial blood glucose elevation in rats and humans [Jpn]. J Jpn Soc Nutr Food Sci, 61: 111-117, 2008.

10) Nakanishi H, Onose S, Kitahara E, Chumchuen S, Takasaki M, Konishi H, Kanekatsu R: Effect of Environmental Conditions on the α-Glucosidase Inhibitory Activity of Mulberry Leaves. Biosci Biotechnol Biochem, 75(12): 2293-2296, 2011.

11) Tormo MA, Gil-Exojo I, Romero TA, Campillo JE: Hypoglycaemic and anorexigenic activities of an amylase inhibitor from white kidney beans (Phaseolus vulgaris) in Wister rats. Br J Nutr, 92: 785-790, 2004.

12) Tsujita T, Takaku T, Suzuki T: Chestnut astringent skin extract, an alpha-amylase inhibitor, retards carbohydrate absorption in rats and humans. J Nutr Sci Vitaminol, 54(1): 82-88, 2008.

13) Tanaka N, Okamura H: Effect of tannin (polyphenol) in a black tea solution on α-amylase activity in saliva [Jpn]. Nippon Kasei Gakkaishi, 40: 587-592, 1989.

14) Turkmen N, Sari F, Velioglu YS: Effects of extraction solvents on concentration and antioxidant activity of black and black mate tea polyphenols determined by ferrous tartrate and Folin-Ciocalteu methods. Food Chemistry, 99(4): 835-841, 2006.

15) Nishiumi S, Bessyo H, Kubo M, Aoki Y, Tanaka A, Yoshida K, Ashida H: Green and black tea suppress hyperglycemia and insulin resistance by retaining the expression of glucose transporter 4 in muscle of high-fat diet-fed C57BL/6J mice. J Agric Food Chem, 58: 12916-12923, 2010.

16) Hara Y, Honda M: The Inhibition of α-amylase by Tea Polyphenols. Agric Biol Chem, 54(8): 1939-1945, 1990.

17) Honda M, Hara Y: Inhibition of rat small intestinal scrase and α-glucosidase activities by tea polyphenols. Biosci Biotech Biochem, 57(1): 123-124, 1993.