Inhibitory effect of tea and flavonoids on the formation of advanced glycation end products.

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Summary
Each tea extract from green tea (Camellia sinensis), pu-ehr tea (Camellia assamica), rooibos tea (Aspalathus linearis) was incubated with a mixture of D-glucose and human serum albumin under physiological conditions (pH 7.4, 37°C). Several flavonoids containing α-G-rutin, G-rutin, G-diosmin, G-naringin, and G-hesperidin were also incubated with a mixture of D-ribose and collagen under the similar conditions. Some of tea extracts and flavonoids showed strong inhibitory effects on the formation of advanced glycation end products (AGE) such as carboxymethyllysine (CML) and pentosidine. As they showed antioxidative potency and radical scavenging activity, tea extracts and flavonoids may be useful tool for inhibition of AGE formation followed by diabetes mellitus and aging.

Keywords
Tea catechin, flavonoid, Maillard reaction, AGE, CML

Introduction
It has been demonstrated that the formation of AGE through the Maillard reaction in vivo is associated with pathogenesis of diabetic complication and aging. The accumulation of AGE is observed in long-lived proteins such as tissue collagen and lens protein. Several recent works proved that glycated protein produce and release superoxide anion radicals and therefore AGE formation closely relates to pathogenesis of the atherosclerosis.

In early stage of the Maillard reaction, an aldelyde group of reducing sugar condensate with an amino group of protein to form Schiff base and then Amadori product (fructosylamine). It changes into 3-deoxy-D-glucoseone or glyoxal followed by the production of AGE in late stage (Fig. 1). The presence of oxygen and oxygen radical (O₂⁻) are not essential, but they show serious effect on the formation of AGE. Several AGE structures have been identified so far. They are N²-(carboxymethyl)lysine(CML), N²-(carboxyethyl)lysine(CEL), imidazolone, pentosidine, crosslines and vesperlysine A.

Tea infusion is one of the most popular beverages in the world. Tea leaves and vegetables contain a variety of polyphenols such as catechin, flavone, flavanone, flavonol. They show high reducing power in vivo and in vitro. Many researches have been reported that green tea catechin show antioxidative, antibacterial, antimutagenic and anticarcinogenis properties, so on.

In this work, we evaluated the inhibitory effect of crude tea extract, catechins and flavonoids on the formation of AGE in vitro. The inhibition potency of tea extracts on CML formation in streptozotocin treated diabetic rats was also determined.

Materials and Methods
Preparation of tea samples
Ten g of pu-ehr tea (China) and rooibos tea (South Africa) leaves were gently boiled in 300 ml of distilled water for 15 min. Ten g of green tea (Shizuoka, Japan) leaves were put into 300 ml of boiling water and
then kept for 5 min at the room temperature. Each tea infusion was filtered on a glass fiber filter and then lyophilized. Ethyl acetate fraction, so-called catechin mixture, was prepared by mixing the infusion with the solvent and evaporated to dryness.

Protein
\[
\begin{align*}
\text{H}_2\text{N-@} & + \\
\text{H}_2\text{COH} & \\
\text{H}_2\text{COH} & \rightarrow \text{Schiff base} \\
\text{H}_2\text{COH} & \rightarrow \text{Amadori product} \\
\text{H}_2\text{COH} & \\
\end{align*}
\]

D-Glucose

\[
\text{ ---> [O]} \rightarrow \text{Glyoxal-adduct}
\]

\[
\text{H}_2\text{N-@} & + \\
\text{CH}_2 & \rightarrow \text{Pentosidine} \\
\text{H}_2\text{COH} & \rightarrow \text{CML}
\]

\[
\text{CHO} \rightarrow \text{CML} \rightarrow \text{Browning Fluorescence} \\
\text{CML} \rightarrow \text{Cross-linking Vesperlysin} \\
\text{AGE receptor}
\]

Fig. 1 Possible Mechanisms of the Maillard Reaction in vivo

Materials and Methods

Inhibition assay of tea extracts and flavonoids on AGE formation in vitro

Sixteen mg of tea crude extract or aminoguanidine were added to 16 ml of 0.2 M phosphate buffer solution (PBS) containing 400 mg of human serum albumin (HSA, Fraction V, Sigma) and 800 mg of D-glucose and then incubated at 37°C. An aliquot of the reaction mixture was taken in periodically and dialyzed against PBS. AGE content was determined by fluometry (Ex 370nm, Em 440nm) and ELISA using

Fig. 2 Inhibitory Effect of Tea Extracts on CML Formation
anti-CML or anti-AGE antibodies. A mixture of collagen and D-ribose was also incubated with and without flavonoids under similar conditions. Aminoguanidine is a reference compound and a trapping reagent for a-oxoaldenylde and inhibit CML-formation from glucose pathway. Antioxidative activity of tea catechins and flavonoids was determined by Fenton reaction-lipid peroxidation method and radical scavenging potency was determined by electron spin resonance.

Results

The inhibitory effect of three kinds of tea extracts and catechin mixture were examined by using D-glucose-HSA system. Green tea, catechin mixture, pu-erh tea showed strong inhibitory effect on AGE formation. CML formation was also inhibited in order of rooibos tea, pu-erh tea, green tea and catechin mixture (Fig.2). These results suggested that other chemicals except catechins are present as inhibitor in green tea. Antioxidative activity of green tea, rooibos tea and catechin mixture was higher than that of pu-erh tea. But aminoguanidine showed extremely low activity. The order of the superoxide anion radical scavenging activity was green tea, pu-erh tea and rooibos tea. The inhibitory effect of 5 kinds of flavonoids on AGE formation was examined by using D-ribose-collagen system. Compared with the control, G-diosmin, α-G-rutin and G-rutin suppressed AGE formation. But G-hesperidine and G-naringin showed weak inhibitory potency. The inhibitory effect of flavonoids on CML formation was shown in Fig.3.

Fig.3 Inhibitory Effect of Flavonoids on CML Formation

Fig.4 Inhibitory Effect of Tea Extracts on AGE Formation in Plasma and Lens Crystallin of Normal or Diabetic Rats

*p<0.01 (vs. Diabetes-Tap water)
The inhibitory tendency was very similar to that of AGE formation. Those flavonoids except G-diosmin showed relatively high antioxidative activity. To test the inhibitory effect of tea extracts on AGE formation in vivo, diabetic rats were used. Diabetes was induced by double intravenous injection of streptozotocin (65mg/kg). 0.05% tea extract solution was given as drinking water for 12 weeks. Body weight and blood glucose level were measured periodically. After dissection, plasma and lens crystalline were collected. In plasma, AGE content was low in the administration groups of pu-erh tea and rooibos tea. But in lens crystalline, the formation of CML was suppressed by administration of pu-erh tea, green tea and rooibos tea (Fig.4).

Conclusion and Discussions
In this experiment, tea extracts containing catechins and several kinds of flavonoids inhibited the formation of AGE, especially of CML. This means these polyphenols may play an important role as inhibitor in the early stage of the Maillard reaction. The inhibitory effects might be to their antioxidative and radical trapping properties. Therefore, we recommend daily intake of tea infusion for preventing age-related disorders containing diabetic complication, atherosclerosis and for regulating aging.

References