Influences of tea infusions and glycyrrhiza solution on both the formation of calcium phosphate precipitates and the calcium chelating ability

Saburo HIDAKA,*[a] Kozo OUCHI,[b] Yuji YAMADA[c] and Yoshizo OKAMOTO[d]

[a]*Department of Dental Hygiene, Fukuoka College of Health Sciences,* [b]*Shinsen Nature Observation Laboratory,* [c]*Smile Dental Clinic,* [d]*Department of Dental Materials, Fukuoka Dental College*

(*Received March 31, 1998. Accepted July 1, 1998.*)

Abstract

The effects of black, oolong, and green tea infusions and glycyrrhiza (Kanzo in Japanese) and tannic acid solutions on both the formation of calcium phosphate precipitates and the calcium chelating ability were studied. All showed strong inhibitory effects on the reaction of calcium phosphate precipitation and transformation. As inhibitory substances, the contents of total polyphenol and fluoride in both infusions and solutions were determined. Although they all contained large amounts of polyphenols, three tea infusions and a tannic acid solution contained great amounts of gelatin-precipitable polyphenols, but the glycyrrhiza solution did not. The order of inhibitory effects on the rate of hydroxyapatite (HAP) transformation was glycyrrhiza solution>three tea infusions>tannic acid solusion. Since the calcium chelating ability which can harm the teeth is extremely strong, the tea infusions which contain great amounts of gelatin-precipitable polyphenols may be unsuitable as an anticalculus agent.

Key words glycyrrhiza, tea infusions, amorphous calcium phosphate, hydroxyapatite, gelatin-soluble or -precipitable polyphenols, fluorides.

Introduction

Since dental calculus can contribute to the progression of gingivitis or periodontal diseases, there have been many efforts to develop an anticalculus agent.[1,2] In recent years, there has been considerable interest in synthetic agents, i.e., sodium etidronate,[3] zinc salts,[4] an oligomer of sulfacrylic acid,[5] and editempa,[6] all of which are useful in the prevention of calculus.[2] However, natural anticalculus agents have not been developed.

Recently, we performed an *in vitro* screening of twenty-three kinds of Chinese traditional (Kampo) medicines for their inhibitory effects on the formation of calcium phosphate precipitates and found that nine medicines have potential as anticalculus agents.[7] Furthermore, we have reported that the polyphenols in several herbs, e.g., glycyrrhiza root, asiasarum root, and cimicifuga rhizome which are constituents of the Kampo medicines and the fractions of rhubarb solution also had inhibitory effects on the formation of calcium phosphate precipitates.[8-10]

Each day an undetermined number of people in the world drink tea and it has been suggested to be effective in decreasing the incidence of both dental caries and periodontal diseases in humans and rats due to tannins (polyphenols), high fluoride contents, and its bacteriocidal effect.[11-15] Teas are an important beverage to prevent oral diseases for on oral hygienist. Therefore, it is of interest to assess the ability of teas as anticalculus agents by comparing them with other substance(s).

In this report, we present the data of the effects of black, oolong, and green tea infusions and an herb, glycyrrhiza solusion on both the formation of calcium

*〒814-0193 福岡市早良区田村 2丁目15番1号
福岡医療短期大学 歯科衛生学教室 日高 三郎
2-15-1, Tamura, Sawara-ku, Fukuoka, 814-0193, Japan

phosphate precipitates and the calcium chelating ability, using a commercial tannic acid as the comparison.

Materials and Methods

Chemical and tea extracts: The dried extract powder of Japanese Pharmacopeia (JP), Glycyrrhiza (Kanzo in Japanese) was supplied by Tsumura & Co., (Tokyo, Japan). The efficiency of extraction was 21.4 % (weight of dried extract powder / weight of Glycyrrhiza used × 100). Ethyleneglycol - bis - (β - aminoethylether) N, N'-tetraacetic acid (EGTA) was purchased from Sigma Chemicals (St Louis, MO, U.S. A.). A 60 % solution of a 1-hydroxyethylidene-1, 1-bisphonate (HEBP) was purchased from Tokyo Kasei (Tokyo, Japan). A tannic acid (Chinese nutgalls) and other reagents were purchased from Katayama Chemicals (Osaka, Japan).

Extracts of black tea (T. J. Lipton, Inc. and PK Co. Ltd., Tokyo, Japan : Product No.49-02203-10126-2) were prepared by immersing one tea bag (average dry weight 2.1 g/bag) in 105 ml distilled water at 100°C for 3 min. Similarly, extracts of oolong tea (Hakata-Chodo Co. Ltd., Japan : Product No.49-73409-00100-7) and green tea (Morio-en Co. Ltd., Japan : Product No. 49-78552-01108-8) were prepared by immersing one tea bag (both average dry weight 5.0 g/bag) in 250 ml distilled water at 100°C for 3 min. The tea bags were removed and the liquids were cooled before use.

Estimation of total polyphenol content : The total polyphenol content in a test solution was measured with the Folin-Ciocalteau reagent.[16] Distilled water (1.0 ml), 0.71M sodium carbonate (0.8 ml), and a test solution (0.2 ml) were mixed and then 0.05 ml of 2N Folin-Ciocalteau reagent was added. After incubation for 15 min at 45°C, the absorbance at 765 nm was determined. Tannic acid was used as the standard.

Precipitation of polymeric polyphenols with gelatin: Polymeric polyphenols (condensed tannins) were precipitated from a test solution by adding an equal volume of gelatin in 5.2M NaCl,[14] followed by the removal of resulting precipitates by filtration using a Sartorius menbrane (Göttingen FRG : pore size 0.3 μm). Polymeric polyphenol concentrations were calculated as the differences between the total polyphenols and the polyphenols remaining in the solution after the gelatin treatment. Previous experiments using black tea showed that the precipitation of polymeric polyphenols increased with higher amounts of gelatin. The precipitation reached a maximum at 1.5 mg/ml of gelatin.[9] In the experiments, gelatin was added at the concentration of 1.5 mg/ml. We chose a concentration of polyphenols ; 334-511 μg/ml.

Fluoride content : Fluoride content was determined with a fluoride-specific ion electrode (94-09, Orion Research Inc., Cambridge, MA, U.S.A.). Ionic strength was adjusted by means of a TISAB buffer.

Assays of amorphous calcium phosphate (ACP) formation and the transformation to hydroxyapatite (HAP) by the pH drop method : The formation of ACP and the trasformation to HAP were measured by the pH drop method.[17] In brief, a pH meter (F-7, Horiba, Japan) with a pH electrode (6028-10T, Horiba, Japan) and a recorder were used for pH measurements.

Two stock solutions, 100 mM $Ca(NO_3)_2$ and 100 mM KH_2PO_4 were prepared in 2 mM Hepes buffer (pH 7.4). To a 1.88 ml of 2 mM Hepes buffer, 60 μl of the calcium stock solution was added. Then, 60 μl of the phosphate stock solution was added to start the reaction. The final concentrations of calcium and phosphate were both 3 mM. The final volume of the reaction mixture was 2 ml. The temperature was maintained at 37±0.1°C and the reaction mixture was stirred continuously.

The formation of ACP and its transformation to HAP *in vitro* occurred in two distinct steps after phosphate (0.75-10 mM) was added to a 3 mM calcium solution at pH 7.4. The reaction was followed acidimetrically by recording pH changes. The pH decrease followed a characteristic pattern.[7,17] We measured the initial rate of pH drop upon addition of phosphate to a calcium buffer solution, and used it as an index of the formation of ACP. The rate of pH changes of the second pH decrease was used as an index for the transformation to HAP.

Tea infusions [about 1/240-1/40 dilutions from a 2 % (w/v) infusions=5-30 μg/ml as a tannic acid], glycyrrhiza solution (3-50 μg/ml as a tannic, acid), and tannic acid solution (5-50 μg/ml) were added to the reaction mixture 5 min before the addition of phosphate. HEBP (10-60 μM) and EGTA (0.125-0.5

mM) or sodium fluoride (25-1000 μM) were also added in the same manner. The reaction rates were converted to the rate of consumption of calcium (parts/10^6/min).[17] The induction time was determined according to the method of Blumenthal *et al.*.[18]

Determination of chelating capacity : Free calcium concentrations in infusions and solutions were determined at 23±0.1°C with a calcium electrode (93-20, Orion Research Inc., Cambring, MA. U.S.A.). A solution containing 3 mM $Ca(NO_3)_2$, 0.08 M KCl, and 20 mM Hepes (pH 7.4) was titrated with three tea infusions and glycyrrhiza solution (10 and 30 μg/ml as a tannic acid), tannic acid solution (10 and 30 μg/ml) and HEBP (10 and 60 μM) or with EGTA (0.5 mM). The changes in free calcium concentration caused by the addition of these infusions and solutions were measured.[7]

Statistics : Date were obtained from 3-5 measurements, and expreseed using mean±standard deviation. Statistical comparisons were made using ANOVA and Scheffé's Test. The difference was considered significant when $p<0.05$.

Results

Total polyphenol contents

As shown in Table I, the average content (mg/ml) of total polyphenol in 2 % (w/v) black, oolong, green tea infusions, and glycyrrhiza solution were not different.

Table I Total polyphenol and fluoride contents in tea infusions, glycyrrhiza, and tannic acid.

	Total polyphenol[a] (mg/ml)	Fluoride[b] (ppm=μg/ml)
None	–	<0.03
Black tea	1.20±0.03	1.65±0.08
Oolong tea	1.30±0.03	2.00±0.05
Green tea	1.29±0.03	0.45±0.01
Glycyrrhiza	1.20±0.03	0.10±0.007
Tannic acid	20±0.5	<0.03

Concentrations used were 2 % (w/v) infusions and solutions.
[a]Values were presented as the amounts (mg/ml) in 2 % (w/v) infusions and solutions using tannic acid as the standard.
[b]Fluoride content was determined with a fluoride specific electrode.

Fluoride contents

As shown in Table I, the order of fluoride contents in 2 % (w/v) infusions was oolong >black > green teas. The fluoride content of glycyrrhiza solution was 1/20-1/4.5 of the contents in tea infusions. That of a commercial tannic acid was <0.03 ppm.

Precipitation of polymeric polyphenols with gelatin

The polyphenol content of the glycyrrhiza solution did not change after the addition of gelatin (Table II), whereas black, oolong, and green tea infusions and the tannic acid solution had much higher gelatin precipitates ; 50, 58, 59, and 85 %, respectively.

The polyphenolic content of 1.5 mg/ml gelatin was 8.4±0.05 μg/ml (as a tannic acid). This value is 1.6-2.5 % of the polyphenol contents of the test infu-

Table II The decrease of polyphenol contents of tea infusions, glycyrrhiza, and tannic acid by the addition of gelatin.

	Addition of gelatin		
	Before (A) (μg/ml)	After (B) (μg/ml)	(B)/Total polyphenol content[a] (%)
Black tea	511±10	261±9.0*	50.3
Oolong tea	468±11	202±8.5*	42.4
Green tea	449±11	186±10*	40.7
Glycyrrhiza	334±9.7	345±9.1	101
Tannic acid	393±10	61.3±7.1*	15.3

Gelatin (final concentration ; 1.5 mg/ml) in 5.2 M NaCl was added to the samples. The concentration range of polyphenols, 334-511 μg/ml, was chosen. After being filtered through a membrane filter (pore size 0.3 μm), a clear liquid was obtained. Polyphenol concentrations before and after gelatin addition were determined.
[a]Total polyphenol content=polyphenol content in the solution+that in gelatin solution.
*Indicates a significant difference ($p<0.05$) from the value before the addition of gelatin.

sions and solutions.

Effects on calcium phosphate precipitation

At the concentrations of 30 μg/ml (as a tannic acid), three tea infusions had greater inhibitory effects on the formation of ACP (7.0-7.7 % of the control) (Table III). However, the glycyrrhiza solution had no inhibitory effects. The tea infusions showed significantly inhibitory effects on both the transformation to HAP (36-43 % of the control) and on the increase of the induction time (2.9 to 3.1 times of the control) (Table III). The glycyrrhiza solution showed inhibitory effects on both the transformation to HAP (32 % of the control) and on the increase of induction time (3.3 times of the control). At the concentrations of 30 and 50 μg/ml, the tannic acid solution had similar inhibitory effects on the formation of ACP (53-63 % of the control), on the transformation to HAP (49-67 % of the control), and on the induction time (1.7 to 2.1 times of the control).

Calcium chelating activity

The calcium complexation of tea infusions and solutions was also examined. As shown in Table IV, at the concentrations of 10 and 30 μg/ml (as a tannic acid), glycyrrhiza showed no significant chelating activities and at the concentration of 30 μg/ml, tannic acid solutions showed significant chelating activities, while black, oolong, and green tea infusions showed extremely strong chelating activities.

Effects of HEBP or EGTA on calcium phosphate precipitation and chelation

Table IV Chelating capability of tea infusions, glycyrrhiza, tannic acid and HEBP.

	Concentration used (as a tannic acid)	Ca^{2+} concentration (mM)
None	–	3.00±0.015
Black tea	10 μg/ml	1.20±0.015*
	30 μg/ml	0.90±0.015*
Oolong tea	10 μg/ml	1.10±0.015*
	30 μg/ml	0.81±0.015*
Green tea	10 μg/ml	1.02±0.015*
	30 μg/ml	0.82±0.015*
Glycyrrhiza	10 μg/ml	2.97±0.015
	30 μg/ml	2.83±0.015
Tannic acid	10 μg/ml	2.80±0.015
	30 μg/ml	2.61±0.013*
HEBP[a]	10 μM (2.0 μg/ml)	2.97±0.011
	60 μM (12 μg/ml)	2.85±0.011
EGTA[b]	0.5 mM (0.19 μg/ml)	2.50±0.1*

Calcium solution (3 mM) containing 0.08 M KCl and 20 mM Hepes (pH 7.4) was titrated with infusions and solutions. The changes in free calcium ion concentration were measured using a calcium electrode.

[a]Abbreviation of 1-hydroxyethylidene-1,1-bisphosphonate. Its concentrations were not expressed as a tannic acid.

[b]Abbreviation of ethylenglycol-bis-(β-aminoethylether) N, N'-tetraacetic acid. Its concentration was not expressed as tannic acid.

*Indicates a significant difference ($p<0.05$) from the control level.

Table III Effects of tea infusions, glycyrrhiza, and tannic acid on the formation of amorphous calcium phosphate (ACP) and its transformation to hydroxyapatite (HAP).

	Concentration used (as a tannic acid)	Ca^{2+} consumption (ppm/min)		Induction time (min)
		ACP	HAP	
None	0	125±12.0	15.0±1.3	14.8±1.5
Black tea	30 μg/ml	9.53±0.83*	5.85±0.55*	45.0±3.5*
Oolong tea	30 μg/ml	9.76±0.90*	5.41±0.54*	46.2±4.1*
Green tea	30 μg/ml	8.76±0.75*	6.45±0.57*	43.5±3.7*
Glycyrrhiza	30 μg/ml	130±10.0	4.81±2.4*	48.2±4.5*
Tannic acid	30 μg/ml	79.3±5.8*	10.1±1.1*	25.5±1.7*
	50 μg/ml	66.2±4.9*	7.35±0.55*	30.5±2.7*

The ACP formation and HAP transformation were measured by the pH drop method. Final concentrations of 3 mM calcium and 3 mM phosphate were used. Additives were added to the reaction mixture 5 min before the addition of 3 mM phosphate. The final volume of the assay solution, which contains 2 mM Hepes (pH 7.4), was 2 ml. The reaction mixture was stirred at 37±0.1°C. Values were presented as the rate of consumption of calcium (ppm/min).

*Indicates a significant difference ($p<0.05$) from the control level.

Table V Effects of HEBP and EGTA on the formation of amorphous calcium phosphate (ACP) and its transformation to hydroxyapatite (HAP).

	Concentration used	Ca^{2+}consumption (ppm/min)		Induction time
		ACP	HAP	(min)
None	0	125±12	15.0±1.3	14.8±1.5
HEBP[a]	10 μM	123±9.3	9.92±0.61*	18.0±1.2
	20 μM	123±8.0	7.81±0.60*	34.7±7.1*
	40 μM	123±8.0	4.61± 0.61*	91.8±8.7*
	60 μM	120±10	2.20± 0.11*	207±13*
EGTA[b]	0.125 mM	119±8.5	13.0±1.3	14.6±1.5
	0.20 mM	122±11	11.8±1.1	15.1±1.4
	0.25 mM	110±9.0	9.20±0.61*	16.5±1.4
	0.50 mM	7.75±0.54*	6.11±0.41*	23.5±2.3*
	2.0 mM	<0.76	ND	>150

Experimental conditions were the same as those shown in Table III.
[a]Abbreviation of 1-hydroxyethylidene-1,1-bisphosphonate.
[b]Abbreviation of ethyleneglycol-bis-(β-aminoethylether) N,N'-tetraacetic acid. ND : not-determined
*Indicates a significant difference ($p<0.05$) from the control levels.

Table VI Effect of sodium fluoride on amorphous calcium phosphate (ACP) formation and its transformation to hydroxyapatite (HAP).

Concentration of NaF (μM)	Ca^{2+}consumption (ppm/min)		Induction time (min)
	ACP	HAP	
0	123±12	13.0±1.3	14.8±1.5
25	123±12	11.7±1.1	20.5±1.5
50	123±11	10.0±0.77*	24.1±1.5*
250	125±11	11.4±1.2	25.3±2.1*
750	121±11	19.9±1.5*	40.0±3.0*
1000	125±11	37.4±2.8*	57.4±4.1*

Experimental conditions were the same as those shown in Table III.
*Indicates a significant difference ($p<0.05$) from the control levels.

HEBP has no inhibitory effects on the ACP formation, but significantly inhibited HAP transformation and elongated the induction time at concentrations of 20-60 μM (Table V). HEBP also showed some chelating activities in 10 and 60 μM (Table IV). EGTA significantly inhibited only HAP transformation at the concentration of 0.25 mM. Above 0.5 mM, it showed significant inhibitory effects on ACP formation, HAP transformation and elongated the induction time (Table V).

Effects of the chelating activity on other measurements

As described above, tea infusions and the glycyrrhiza solution had significant or very strong calcium chelating abilities at the concentrations of 10 and 30 μg/ml. Therefore, we examined whether this activity would effect calcium phosphate precipitation. The chelating activities of three tea infusions at the concentrations of 10 and 30 μg/ml (as a tannic acid) were equivalent to 1.8-1.98 and 2.10-2.19 mM EGTA, respectively (Table IV). Those of glycyrrhiza solution were equivalent to 0.03-0.17 mM EGTA (Table IV). AT the concentrations of 1.8-2.19 mM EGTA, it showed high inhibitory effects on the calcium phosphate precipitation: EGTA inhibited only the rate of HAP transformation at the concentration of 0.25 mM (Table V).

Comparison with HEBP

The inhibitory effect on the HAP transformation was compared between HEBP (10-60 μM) and tea infusions and solutions (3-50 μg/ml ; as a tannic acid). As shown in Tables III and IV, a 50 % inhibition of HAP transformation was attained with concentrations of 4.5 μg/ml (=22 μM) of HEBP, 21-25 μg/ml of tea infusions, 13 μg/ml of glycyrrhiza, and 49 μg/ml of tannic acid solutions. This indicated that the effectiveness of tea infusions, glycyrrhiza, and tannic acid solutions was about 1/5-1/6, 1/3, and 1/11 of that of HEBP, respectively in terms of the activity per weight.

Effects of fluoride on calcium phosphate precipitation

As shown in Table VI, the addition of 25 μM (=0.88 ppm as fluorine ion) sodium fluoride had no effects on the rate of HAP transformation and on the induction time. However, the addition of 50 μM (=1.75 ppm as fluorine ion) sodium fluoride decreased the rate of HAP transformation (77 % of the control) and elongated the induction time (1.6 times of the control). At higher concentrations (750-1000 μM), sodium fluoride increased the rate of HAP transformation by 53-188 % with a further increase of induction time (2.7-14 times longer than the control).

Discussion

Tannins whose hydrolyzed product is a tannic acid, are one of the polyphenols. Furthermore, polyphenols (tannins) are classified into two groups by means of gelatin addition, i.e., hydrolyzable (gelatin-soluble) and polymeric (gelatin-precipitable) polyphenols (condensed tannins) and are present in a wide variety of plants used for medicine and food.[19] In this study, it was found that 2 % (w/v) concentration of both tea infusions and glycyrrhiza solution contained 6-6.5 % polyphenols (Table I). However, glycyrrhiza solution produced no amounts of polymeric polyphenols with gelatin (see Table II), whereas three tea infusions produced much higher amounts of polymeric polyphenols (Table II).

A chemically synthesized agent, HEBP has been proven to be effective in decreasing the dental calculs in the rat and human,[3,4,20] but it may be toxic at the effective dose, because it could affect bone turnover and mineralization.[21] The effects in children, where bone mineralization is actively occurring, are of some concern. Therefore, a safer anticalculus agent is required.

Compared with 50 % inhibition of the HAP transformation, the inhibitory activity of tea infusions and glycyrrhiza solution was about 1/5-1/6 and 1/3 of that of HEBP, respectively. This strongly suggests that tea infusions and glycyrrhiza solution may be useful anticalculus agents derived from a natural source. However, the chelating ability of tea infuions is stronger compared to that of glycyrrhiza solution (Table IV). We found that, at the concentration of 30 μg/ml (as a tannic acid), tea infusion virtually inhibited the formation of calcium phosphate precipitates as shown by the reduction of the rate of ACP formation (Table III). Their strong chelating ability was suggested from an inhibitory pattern comparable to concentrations of 0.25 mM EGTA (Table V). Tea infusions may inhibit the calcium phosphate precipitation entirely by sequestration of calcium ions from the environmental solutions. Since the calcium chelating ability could affect the teeth especially cementum, tea infusions with high amounts of gelatin-precipitable polyphenol may not be suitable as anticalculus agents. The major polyphenolic compounds from tea infusions are a group of cathechins.[19,22] These polyphenolic compounds may exhibit extremely strong chelating activity. It has been known that the commercial tea is a potent source of natural fluoride whose action together with polyphenols (tannins) affect the cariogenic bacterial functions.[11] Epidemiological studies in tea-drinking countries suggest that changes in the basic diet including tea-drinking habits affect the caries rate dramatically.[23] Therefore, the extremely strong chelating activity of the tea infusions may regrettably be a fault to use for the anticalculus agent. In contrast, glycyrrhiza, as well as asiasarum root and cimicifuga rhizome, contain 90-100 % gelation-soluble polyphenols which have weak calcium chelating ability and strong inhibitory effect on the calcium phosphate precipitation.[8,9] This indicates that these herbal solutions may be more suitable as anticalculus agents.

The distinctive flavors associated with the three main classes of tea, i.e., black, oolong, and green teas result from the degree of tannin oxidation, that is allowed to take place following plucking.[11] However, both contents of total and gelatin-precipitable polyphenols were almost the same in all tea infusions and those of fluoride were slightly varied from 0.45 to 2.0 ppm (Table I).

Both polyphenols and fluoride content influence the formation of glucan, not only through the action of polyphenol on the glucosyltransferase, dextran sucrase, but also affecting the growth of the microorganism and the storage of polysaccharide.[11] Thus teas with high fluoride and polyphenol content may affect the cariogenic bacterial functions. Furthermore, both the polyphenol and fluoride content influence the formation of oral calcium phosphate precipitates, e.g., calculus formation and remineralization of enamel.[17] In 2 % (w/v) black tea infusion, the ratio of polyphenol/fluoride contents is 1200/1.65=727 (see Table I). Such a large ratio for polyphenol may easily hide the promoting effect on HAP crystallization produced by fluorides (Table VI) in tea infusions. However, for the interpretation of synergistic effect between polyphenol and fluoride, further elucidation is required.

The effect of fluoride ions was difficult to interpret ; it inhibited the rate of HAP trasformation at the

concentration of 50 μM, but promoted it at the concentration above 750 μM (Table VI). Concerned with the dual effect of fluoride, Meyer and Nancollas[24] have reported this phenomenon in lower concentrations, using a different method. The effect of the fluoride on the crystal growth of HAP is complicated; it appears to inhibit growth at a small concentration (<10 μM) but enhances growth at a greater concentration (>10 μM).[24]

In summary, we studied the influences of black, oolong, and green tea infusions and glycyrrhiza solution on the formation of calcium phosphate precipitates. They all showed strong inhibitory effects with or without strong calcium chelating activities. Because of their extremely strong calcium chelating ability, gelatin-precipitable polyphenols which are highly contained in tea infusions may be unsuitable as anticalculus agents.

Acknowledgements

This work was supported in part by a research grant from Tsumura & Co., Japan.

和文抄録

紅茶，ウーロン茶，緑茶浸出液，あるいは甘草とタンニン酸溶液がリン酸カルシウム沈殿物形成とカルシウムキレート能に与える効果につき研究した。これら浸出液と溶液はリン酸カルシウム沈殿物の形成と転換反応とに強い抑制効果を示した。抑制物質として，総ポリフェノールとフッ化物含量を測定した。すべての浸出液と溶液が大量のポリフェノールを含有するなかで，三種類の茶浸出液とタンニン酸溶液は大量のゼラチン沈殿ポリフェノールを含有し，甘草溶液はそれを含有していなかった。ハイドロキシアパタイトへの転換能に対する抑制効果は，甘草溶液＞三種類の茶浸出液＞タンニン酸溶液の順であった。しかしながら，歯牙に害を与えるカルシウムキレート能が非常に強かったので，ゼラチン沈殿ポリフェノールを大量に含有する茶浸出液は抗歯石剤としては不適当であろう。

References

1) Mandel, I. D. and Gaffar, A. : Calculus revisited : a review. *J. Clin. Periodontol.* **13**, 249-257, 1986.

2) Mandel, I. D. : Calculus formation and prevention : an overview. *Comp. Cont. Educ. Dent.* 8 (suppl), 235-241, 1987.

3) Briner, W. W. and Francis, M. D. : *In vitro* and *in vivo* evaluation of anti-calculus agents. *Calcif. Tissue Res.* **11**, 10-22, 1973.

4) Suomi, J. D., Horowitz, H. S., Barbano, J. P., Spolsky, V. W. and Heifetz, S. B. : A clinical trial of a calculus inhibitory dentifrice. *J. Periodontol.* **45**, 139-145, 1974.

5) Gaffar, A, Niles, H. P. and Davis, C. D. : Evalution of an oligomer or an origomer plus cetyl pridinium chloride against plaque, stain, calculus, and gingivitis. *J. Dent. Res.* **60**, 1432-1439, 1981.

6) Gaffar, A., Moreno, E.C., Muhleman, H. R. and Niles, H. P. : Effects of EDITEMPA on dental calculus and caries formation *in vivo*. *Calcif. Tissue Int.* **35**, 362-365, 1983.

7) Hidaka, S., Abe, K., Takeuchi, Y., Liu, S. Y. : Inhibition of the formation of oral calcium phosphate precipitates : beneficial effects of Chinese traditional (Kampo) medicines. *J. Periodont. Res.* **28**, 27-34, 1993.

8) Hidaka, S., Okamoto, Y., Abe, K., Suekawa, M. and Liu S. Y. : Effective fractions of a Chinese traditional (Kampo) medicine, Rikko-san and its herbal constituents on the formation of calcium phosphate precipitates. *J. Traditional Med.* **11**, 106-113, 1994.

9) Hidaka, S., Nakajima, K., Suekawa, M. and Liu, S. Y. : Saliva and gelation form precipitates with some Chinese traditional herbs and formulas. *Int. J. Ori. Med.* **20**, 169-176, 1995.

10) Hidaka, S., Nishimura, H., Nakajima, K. and Liu, S. Y. : Effects of a rhubarb (*Rhei Rhizome*) solution and its fractions on the formation of calcium phosphate precipitates. *J. Periodont. Res.* **31**, 408-413, 1996.

11) Elvin-Lewis, M., Vitale, M. and Kopjas, T. : Anticariogenic potential of commercial teas. *J. Prev. Dent.* **6**, 273-284, 1980.

12) Ryu, E. : Prophylactic effect of tea on pathogenic micro-organism infection to human and animals. (1) Growth inhibitive and bacterocidal effect of tea on food poisoning and other pathogenic enterobacterium *in vitro*. *Int. J. Zoonoses*. **7**, 164-170, 1980.

13) Rosen, S., Elvin-Lewis, M., Beck, F.M. and Beck, E. N. : Anticariogenic effects of tea in rats. *J. Dent. Res.* **63**, 658-660, 1984.

14) Kashket, S., Paolino, V. J., Lewis, D. A. and van Houte, J. : *In-vitro* inhibition of glucosyltransferase from the dental plaque bacterium *Streptococcus mutans* by common beverages and food extracts. *Arch. Oral Biol.* **30**, 821-826, 1985.

15) Otake, S., Makimura, M., Kuroki, T., Nishihara, Y. and Hirasawa, M. : Anticaries effects of polyphenolic compounds from Japanese green tea. *Caries Res.* **25**, 438-443, 1991.

16) Hoff, J. E. and Singleton, K. I. : A method for determination of tannins in foods by means of immobilized protein. *J. Fd. Sci.* **42**, 1566-1569, 1977.

17) Hidaka, S., Abe, K. and Liu, S. Y. : A new method for the study of the formation and transformation of calcium phosphate precipitates: Effects of several chemical agents and Chinese folk medicines. *Archs. Oral Biol.* **36**, 49-54, 1991.

18) Blumenthal, A. W., Betts, F. and Posner, A. S. : Nucleotide stabilization of amorphous calcium phosphate. *Mat. Res. Bull.* **10**, 1055-1060, 1975.

19) Okuda, T., Yoshida, T. and Hatano, T. : New methods for analyzing tannins. *J. Nat. Prod.* **52**, 1-31, 1989.

20) Hidaka, S., Abe, K. and Liu, S. Y. : *In vitro* and *in vivo* evaluations of Chinese traditional (Kampo) medicines as anticalculus agents in the rat. *Archs. Oral Biol.* **38**, 327-335, 1993.

21) Baumann, J. M., Bisaz, S., Fleisch, H. and Wacker, M. : Biochemical and clinical effects of ethan-1-hydroxy-1, 1'-diphosphonate in calcium nephrolithiasis. *Clin. Sci. Molec. Med.* **54**, 509-516,

1978.

22) Hattori, M., Kusumoto, I. T., Namba, T., Ishigami, T. and Hara, Y. : Effect of tea polyphenols on glucan synthesis by glucosyltransferase from *Streptococcus mutans*. *Chem. Pharm. Bull.* **38**, 717-720, 1990.

23) Infirri, J. S. and Barmes, D.E. : Epidermiology of oral diseases—differences in national problems. *Int. Dent. J.* **29**, 183-190, 1979.

24) Meyer, J. L. and Nancollas, G. H. : Effect of stannous and fluoride ions on the rate of crystal growth of hydroxyapatite. *J. Dent. Res.* **51**, 1443-1450, 1972