FEBS Letters 433 (1998) 44–46 FEBS 20642

Effect of green and black tea supplementation on lipids, lipid oxidation and fibrinogen in the hamster: mechanisms for the epidemiological benefits of tea drinking

Joe A. Vinson*, Yousef A. Dabbagh

Chemistry Department, University of Scranton, Scranton, PA 18510, USA

Received 11 May 1998; revised version received 6 July 1998

Abstract There is considerable epidemiological evidence that tea drinking lowers the risk of heart disease. However, the mechanism by which tea can be protective is unknown. Hamsters were fed a normal or high cholesterol diet for 2 weeks and drank green or black tea ad libitum. The plasma lipid profile was significantly improved by both teas compared to controls. Also in vivo lipid oxidation as measured by plasma lipid peroxides and LDL+VLDL oxidizability were significantly decreased by the teas. In the normal fed tea groups fibrinogen was decreased but not in the high cholesterol groups. Green tea was significantly more effective than the black tea. These results show in the hamster model that black and green tea improve the risk factors for heart disease by both hypolipemic and antioxidant mechanisms and possibly a fibrinolytic effect.

Key words: Cholesterol; Triglyceride; Atherogenic index; Fibrinogen; Lipid peroxide; Lipid oxidation

1. Introduction

Tea, prepared from the leaves of *Camella sinensis*, is the most popular beverage in the world except water. Black tea, made from the mild oxidation of green tea leaves, amounts to 80% of world tea production [1]. Flavonoids are a group of polyphenols present in vegetables, fruits and beverages such as tea and wine. Tea is the major source of dietary flavonoids in Japan (7 cups/day), Holland (4 cups/day) and a minor source of the flavonoids in the US diet at 0.5 cups/day [2]. Flavonoid intake is inversely associated with mortality from coronary artery disease in a cross-cultural seven country epidemiological study [2]. A Japanese study found green tea consumption was associated with decreased cholesterol and triglyceride and an increased proportion of HDL [3].

Green tea and black tea are both high in catechins. These compounds are powerful antioxidants, capable of rapid reduction of superoxide radical and alkyl peroxy radicals. Catechins may also repair vitamin E radicals [4]. Such potent antioxidant ability may be important in inhibiting the in vivo oxidation of LDL and VLDL and the subsequent atherogenesis. Catechins have been found to be the most potent group of antioxidants for inhibiting in vitro lower density lipoprotein oxidation by cupric ion [5]. Catechins and other flavonoids were found to incorporate in lower density lipoproteins isolated from spiked plasma [6]. Theaflavins incorporated in LDL were better than catechins in inhibiting LDL oxidation by macrophages [7].

The mechanism of tea's benefit for heart disease as found from the epidemiological studies is not known. Nor is the relative benefit of green vs. black tea known. This study was designed to ascertain and compare the effect of the consumption of green and black tea on heart disease risk factors in normal and cholesterol-fed hamsters.

2. Materials and methods

2.1. Chemicals

Teas were obtained from Thomas J. Lipton Company (Englewood Cliffs, NJ) and were kept in sealed plastic bags until use. They were made up as 1.25% solutions in boiling water and 16 g of Sweet N'Low was added/liter to mask the teas' bitter taste. The total phenol concentrations in the teas were measured by the Folin-Cocialteu reagent (Sigma) using catechin as a standard and were 8.88 mM and 9.17 mM for black and green tea, respectively.

2.2. Animals and diets

Male, Syrian golden hamsters (Charles River Labs, Wilmington, MA) weighing 115–150 g were divided into six groups of six animals each and three animals to a cage. The animals were on a wood chip bedding in a climate and light-controlled animal facility. Animals had free access to food in the form of brownies. Normal groups ate brownies from chow to which was added water alone at a ratio of 600 ml/kg of chow. Cholesterol-fed animals had 0.2% cholesterol and 10% coconut oil melted and mixed with 600 ml/kg water and chow, pressed flat, frozen and cut into brownies [8]. The normal control group received the normal chow and water with Sweet N'Low and the normal experimental groups normal chow and green or black tea with Sweet N'Low. The high cholesterol groups received the high fat chow and the appropriate drink. Food and drink were given ad libitum for the study that lasted 14 days. After 18 h fast the animals were killed by pentobarbital overdose and plasma collected and frozen at −90°C until analysis.

2.3. Analysis

Total cholesterol, HDL and triglycerides were assayed by Sigma enzyme kits. HDL and LDL+VLDL were isolated by affinity column chromatography (Isolabs, Cincinnati, OH). The atherogenic index was calculated from the following formula: atherogenic index = (total cholesterol−HDL)/HDL. Fibrinogen was measured by a Sigma kit using the thrombin clotting time method. Lipid peroxides in plasma were assayed by a fluorimetric thiobarbituric acid method using tetramethoxypropane as the standard [9]. LDL+VLDL oxidation was done under standard conditions using a published procedure [10]. The protein concentration was measured using a Sigma kit and adjusted to 70 μg/ml and the lipoproteins oxidized at pH 7.4 with 25 μM cupric ion in phosphate buffered saline at 37°C. Conjugated dienes were followed by measuring the absorbance at 234 nm every 15 min vs. a blank containing cupric ion in PBS. Graphs of absorbance vs. time were used to calculate the lag phase defined as the intercept of straight lines drawn along the slopes of the lag and propagation phases. The concentration of conjugated dienes in the samples were calculated from the literature value of $\varepsilon = 29\,500$ [11]. Results were compared statistically using a student's *t*-test or Mann-Whitney rank sum test.

*Corresponding author. Fax: +1 (717) 941-7510.
E-mail: vinson@tiger.uofs.edu

Abbreviations: LDL, low density lipoproteins; VLDL, very low density lipoproteins; HDL, high density lipoproteins

PII: S0014-5793(98)00880-1

3. Results

3.1. Effect of teas on plasma lipids, lipoproteins and fibrinogen

There was no significant difference in food consumption or weight gain among any of the groups during the 2-week study. Both tea groups consumed significantly more fluid than their respective control group and the black tea groups drank more fluid than their green tea counterparts ($P < 0.02$). The results of the biochemical analyses are shown in Table 1.

In the normal chow-fed animals the cholesterol was decreased 17% by black tea and 27% by green tea, both significant. The black tea did not change HDL while green tea raised HDL 10%, a significant change. Triglycerides were decreased significantly by black tea 21% and 36% by green tea. The atherogenic index was significantly decreased by both teas; black tea 34% and green tea 71%. Black tea significantly decreased fibrinogen 42% and green tea decreased it 38%. Both teas beneficially affected these heart disease risk factors in normal animals and the green tea was significantly better than the black tea with respect to cholesterol, HDL, triglycerides and the atherogenic index.

The cholesterol-fed hamsters experienced an increase in cholesterol and a decrease in the % of cholesterol as HDL as has been shown previously [11]. Cholesterol was significantly lowered by black tea 20% and by green tea 28%. HDL was increased significantly by both teas; black tea 35% and green tea 88%. Both teas significantly decreased triglycerides; black tea 20% and green tea 48%. Black tea lowered the atherogenic index 48% and green tea lowered it 73%. Neither tea significantly affected the elevated fibrinogen of the cholesterol-fed animals. Both teas significantly improved the lipid profile and as in the normal chow groups, green tea was significantly better than black in improving the lipid profile.

3.2. Effect of teas on plasma lipid peroxides and lower density lipoprotein oxidation

As shown in Table 1 and illustrated in Fig. 1, both teas were powerful inhibitors of plasma lipid peroxides and LDL+VLDL oxidizability. Green tea was a significantly better in vivo antioxidant than black tea in both the normal and cholesterol-fed groups. As expected the cholesterol feeding produced an increase in plasma lipid peroxides and LDL+VLDL oxidizability; the latter as shown by the decrease in lag time and the increased maximal oxidation rate. Both teas significantly lengthened the lag phase in both the normal and cholesterol-fed groups and green tea was significantly better than black tea. Green tea increased the normal animals lag time 71% and the high cholesterol animals 63%. In fact the green tea increased the lag time of the cholesterol-fed animals to the extent that it was not significantly different from the normal control group. The maximal oxidation rate in the high cholesterol-fed animals was only decreased significantly by green tea. On the other hand only black tea significantly decreased the maximal rate of oxidation in the normal group.

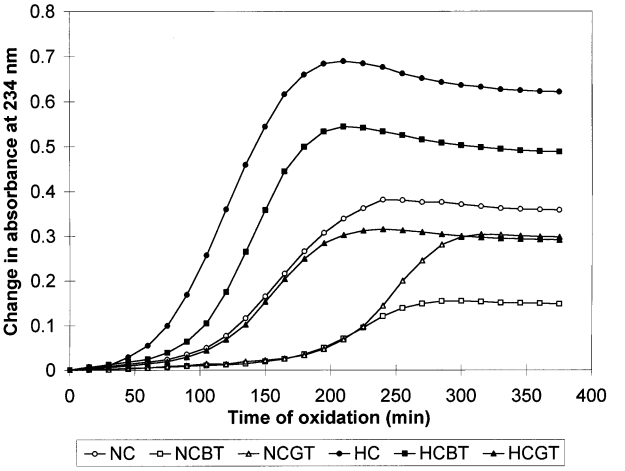

Fig. 1. Average LDL+VLDL oxidation curves of normal and cholesterol-fed hamsters given tea for 2 weeks. NC, normal cholesterol control; NCBT, normal cholesterol+black tea; NCGT, normal cholesterol+green tea; HC, high cholesterol control; HCBT, high cholesterol+black tea; HCGT, high cholesterol+green tea.

4. Discussion

The male Syrian Golden hamster is an excellent model for the study of lipid and lipoprotein metabolism and it has been used in this study. Short-term tea supplementation at a strength used for human consumption produced large improvements in the lipid profile of both normal and cholesterol-fed animals. A Japanese study found the same results with green tea catechins given to cholesterol-fed rats [12]. In epidemiological studies both green and black tea drinking have been associated with decreased plasma cholesterol and a decreased atherogenic index [13,14]. Our animal study confirms this association. In addition we have found that both teas decrease fibrinogen in normal animals. Elevated fibrinogen levels are a well established independent risk factor for coro-

Table 1
Effect of tea drinking on plasma lipids and fibrinogen for hamsters fed a normal or high cholesterol diet for 2 weeks (mean ± S.D.)

	Normal cholesterol groups			High cholesterol groups		
	Control	Black tea	Green tea	Control	Black tea	Green tea
Cholesterol (mg/dl)	110 ± 5	91 ± 3	80 ± 2**	183 ± 8	146 ± 3*	130 ± 4**
HDL (mg/dl)	58 ± 3	58 ± 3	64 ± 3**	26 ± 1	37 ± 3*	49 ± 3**
Triglycerides (mg/dl)	159 ± 3	126 ± 3	102 ± 5**	292 ± 11	234 ± 7*	200 ± 9**
Cholesterol−HDL/HDL	0.85 ± 0.11	0.56 ± 0.05*	0.25 ± 0.07**	6.15 ± 0.05	2.91 ± 0.32*	1.69 ± 0.21**
Fibrinogen (mg/dl)	165 ± 9	95 ± 9*	102 ± 12*	194 ± 12	186 ± 9	187 ± 13
Lipid peroxides (μM)	0.83 ± 0.09	0.57 ± 0.09*	0.36 ± 0.09**	2.54 ± 0.28	1.86 ± 0.18*	1.29 ± 0.15**
Lag time (min)	119 ± 11	177 ± 8*	204 ± 3**	72 ± 4	87 ± 13	117 ± 6**
Diene oxidation rate (nm/mg) protein min)	1.5 ± 0.6	0.8 ± 0.2**	1.7 ± 0.9	3.2 ± 0.2	3.0 ± 0.4	1.7 ± 0.4**

Cholesterol−HDL/HDL.
*Significantly different from control, $P < 0.05$.
**Significantly different from the control and the corresponding tea group, $P < 0.05$.

nary, cerebral and peripheral vascular disease. Fibrinogen strongly affects blood coagulation, blood rheology and platelet aggregation and has direct effects on the vascular wall [15]. In the normal groups there was a positive correlation of cholesterol with fibrinogen, Pearson product moment correlation coefficient 0.7997, $P < 0.0001$. A possible mechanism for teas' effect on fibrinogen is found in a recent study with epigallocatechin gallate, the major polyphenol in green tea and a component of black tea. This compound and other gallates were found to bind to fibrinogen in human serum [16]. The binding may inhibit the function of fibrinogen. This beneficial effect of tea needs to be further investigated in both animal and human studies.

Although it is difficult to dissociate the lipid lowering effect from the antioxidant effect, it appears that both green and black tea are powerful in vivo inhibitors of lipid oxidation. We have found (unpublished results, submitted for publication) that both green and black tea polyphenols bind to lower density lipoproteins when spiked in plasma. This incorporation, subsequent to tea polyphenol absorption into the plasma, would explain the increase in lag time after consumption of both teas in the current study. A Japanese group has also found that black tea consumption (750 ml/day) for 4 weeks prolonged the lag time of LDL oxidation in normocholesterolemic men [6]. As is apparent from our study, hypercholesterolemic human subjects should also be investigated.

The present hamster study shows that teas in the concentration normally drunk by humans are hypolipemic, antioxidant and possibly fibrinolytic agents, and thus provides the mechanism to explain teas' epidemiological benefits for heart disease. Further long-term dose-response studies should be done to determine the effect of teas on normal hamsters and atherosclerosis in cholesterol-fed hamsters. The present study suggests in the hamster model that both black and green tea decrease risk factors for heart disease in both normal and hypercholesterolemic animals.

Acknowledgements: The financial assistance of Thomas J. Lipton, Co. is gratefully acknowledged.

Nicole Sandone and John Proch are thanked for animal care and feeding.

References

[1] Graham, H.N. (1992) Prev. Med. 21, 334–350.
[2] Hertog, M.G.L., Kromhout, D., Arvanis, C., Blackburn, H., Buzina, R., Fidanza, F., Giampaoli, S., Jansen, A., Merlotti, A., Nedjkovic, S., Pekkarinen, M., Bozidar, S.S., Toshima, H., Feskens, F.J.M., Hollman, P.C.H. and Katan, M.B. (1995) Arch. Intern. Med. 155, 381–386.
[3] Imai, K. and Nakachi, K. (1995) Br. Med. J. 310, 693–696.
[4] Jovanovic, S.V., Steenken, S., Hara, Y. and Simic, M.G. (1996) J. Chem. Soc. Perkin Trans. 2, 2497–2504.
[5] Vinson, J.A., Dabbagh, Y.A., Serry, M.M. and Jang, J. (1995) J. Agric. Food Chem. 43, 2800–2802.
[6] Vinson, J.A., Jang, J., Dabbagh, Y.A., Serry, M.M. and Cai, S. (1995) J. Agric. Food Chem. 43, 2798–2799.
[7] Ishikawa, T., Suzukawa, M., Ito, T., Yoshida, H., Ayaori, M., Nishiwaki, M., Yonemura, A., Hara, Y. and Nakamura, H. (1997) Am. J. Clin. Nutr. 66, 261–266.
[8] Nicolosi, R.J. (1991) Atherosclerosis 91, 35–49.
[9] Lepage, G., Munoz, G., Champagne, J. and Roy, C.C. (1991) Anal. Biochem. 277, 277–283.
[10] Vinson, J.A. and Hontz, B.A. (1995) J. Agric. Food Chem. 43, 401–403.
[11] Esterbauer, H. and Jürgens, G. (1993) Curr. Opin. Lipidol. 4, 114–124.
[12] Hara, Y. (1994) in: C.-T. Ho, T. Osawa, M.-T. Huang and R.T. Rosen (Eds.), Food Phytochemicals for Cancer Prevention II, American Chemical Society, Washington, DC, pp. 35–50.
[13] Ito, Y., Sasaki, R., Shinohara, R., Yagyu, S., Suzuki, S. and Aoki, K. (1994) Med. Biol. 129, 21–24.
[14] Kono, S., Shinchi, K., Ikeda, N., Yanai, F. and Imanishi, K. (1992) Prev. Med. 21, 526–531.
[15] Ernst, E. (1993) Atherosclerosis 100, 1–12.
[16] Sazuka, M., Itoi, T., Suzuki, Y., Odani, S., Koide, T. and Isemura, M. (1996) Biosci. Biotech. Biochem. 60, 1317–1319.