ELSEVIER

Atherosclerosis 193 (2007) 86–93

ATHEROSCLEROSIS

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A green tea extract lowers plasma cholesterol by inhibiting cholesterol synthesis and upregulating the LDL receptor in the cholesterol-fed rabbit

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Received 5 June 2006; received in revised form 2 August 2006; accepted 14 August 2006
Available online 12 September 2006

Abstract

Green tea extracts enriched in catechins decrease plasma cholesterol in hamsters, mice and rats. The aims of this study were to determine whether a catechin-enriched extract of green tea could lower plasma cholesterol in the cholesterol-fed rabbit and to determine the mechanism of action. Four groups of six New Zealand White rabbits were initially made hypercholesterolaemic by feeding a 0.25% (w/w) cholesterol diet for 2 weeks before the diet was supplemented with a catechin extract from green tea at 0, 0.5, 1 or 2% (w/w) for 4 weeks. Administration of the crude catechin extract from green tea significantly ($p < 0.05$) lowered cholesterol in plasma (−60%), VLDL + IDL (−70%), LDL (−80%), liver (total by −25% and unesterified by −15%) and aorta (−25%) compared to control. There was a significant reduction in the cholesterol synthesis index (−60%) and a significant increase in hepatic LDL receptor activity (+80%) and protein (+70%) but there was no change in the intrinsic capacity to absorb cholesterol from the intestines. These results suggest that green tea catechins lowered plasma, liver and aortic cholesterol in the cholesterol-fed rabbit by lowering cholesterol synthesis and upregulating the hepatic LDL receptor.

Keywords: Green tea; LDL receptor; Cholesterol synthesis; Cholesterol absorption; Lathosterol; Phytosterols; Aorta

1. Introduction

Green tea is a widely consumed beverage brewed from the plant species '*Camellia sinensis* (L.) O. Kuntze'. It contains an abundance of naturally occurring polyphenols called catechins of which epigallocatechin gallate (EGCG) is the most prevalent. Epidemiological studies [1–3] have found that drinking between 5 and 10 cups of green tea per day is associated with lower plasma cholesterol concentrations. Intervention studies in rats, mice and hamsters have also found that green tea or green tea extracts enriched in catechins exhibit hypocholesterolaemic effects [4–8]. In contrast, Tijburg et al. [9] found that a green tea extract, included in the drinking water, did not significantly decrease cholesterol concentrations in the cholesterol-fed hypercholesterolaemic rabbit.

Inhibition of cholesterol absorption has been proposed as a mechanism to explain the cholesterol lowering effects of green tea. This is because the faecal excretion of total lipids and cholesterol were found to be higher in animals consuming green tea extracts [4,5,8]. The EGCG has also been observed to inhibit the uptake of ^{14}C-cholesterol from the intestine [10]. This apparent reduction in intestinal cholesterol absorption has been ascribed to EGCG reducing the solubility of cholesterol into mixed bile salt micelles [11]. It has also been found that hamsters and rats fed green tea extracts had increased faecal excretion of bile acids [7,8].

This apparent decrease in cholesterol absorption and bile acid reabsorption by green tea should lead to a reduction

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doi:10.1016/j.atherosclerosis.2006.08.033

in liver cholesterol concentrations. In order to compensate for this it would be expected that LDL receptor activity and cholesterol synthesis in the liver would increase [12]. These effects have been noted in studies using inhibitors of cholesterol absorption such as tiqueside [13] and inhibitors of intestinal reabsorption of bile acids such as cholestyramine [14]. The increase in the LDL receptor can mediate the lowering of plasma cholesterol by enhancing the uptake of LDL cholesterol from the circulation. However, the cholesterol lowering potential of these agents can be offset by an increase in cholesterol synthesis [12].

Studies in vitro [15–17] have provided evidence that green tea extracts and its catechin constituents can upregulate the LDL receptor and modulate cholesterol metabolism in HepG2 cells. Indirect evidence has also been found in vivo. When rats were fed EGCG, the removal of intravenously injected ^{14}C-cholesterol from the plasma was enhanced [10]. This increase in the plasma clearance of cholesterol may have been due to upregulation of the LDL receptor as it is the main mechanism by which the sterol is removed from the circulation [12]. There is, however, no study to date that has investigated the effects of green tea or green tea extracts on the LDL receptor in vivo.

Little is known about the effects of green tea on cholesterol synthesis. Studies have found no effect of green tea on the "in vitro" activity of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase [7,8]. The HMG-CoA reductase enzyme catalyses the rate-limiting step in cholesterol biosynthesis, but measurement of its activity "in vitro" may not always reflect the level of cholesterol synthesis. For example, treatment with inhibitors of cholesterol synthesis (e.g. the statins) has been found to elevate, not decrease, "in vitro" HMG-CoA reductase activity [18,19]. More direct measures of cholesterol synthesis such as the incorporation of tritium in cholesterol using tritiated water [20] and the plasma ratio of lathosterol to cholesterol have confirmed that whole body cholesterol synthesis is, in fact, lowered with statin treatment. This is despite often marked increases in "in vitro" HMG-CoA reductase activity [19].

The aims of this study were to determine if a crude catechin extract from green tea could lower plasma cholesterol concentrations in the cholesterol-fed hypercholesterolaemic rabbit and to determine if a green tea extract could upregulate the LDL receptor and increase cholesterol synthesis.

2. Materials and methods

2.1. Catechin extract

The crude catechin extract was prepared from commercially available "Special Gunpowder" green tea, packaged by the China National Native Products and Animal By-products Import and Export Corporation, Zhejiang Tea Branch, China. The method used was based on the method of Huang et al. [21]. Briefly, 15 kg of green tea was extracted with 3 volumes (v/w) of methanol at 50 °C for 3 h. Solvent was removed from the extract using a reduced pressure rotary evaporator. The residue was dissolved in 2 volumes of water (v/w) at 50 °C and extracted twice with equal volumes of hexane (v/v) and once with an equal volume of chloroform (v/v). The remaining aqueous phase was then extracted once with an equal volume of ethyl acetate (v/v) which extracts the polyphenolic compounds including the catechins. The ethyl acetate was then evaporated, the residue redissolved in the minimum amount of warm water (50 °C) and freeze dried. The extract contained at least 58% (w/w) catechins and the composition of the measured constituents were: 30% EGCG, 21% ECG, 10% caffeine, 6% moisture, 4% EGC, 2% GCG and 0.5% theanine.

2.2. Animal study

Twenty-four (4 month old) male New Zealand White rabbits (IMVS, Gillies Plains, SA, Australia) were housed in individual cages at the CSIRO Health Sciences and Nutrition animal facility (Kintore Avenue, Adelaide, SA, Australia). Ethics approval for the study was obtained from the University of Adelaide and CSIRO Health Sciences and Nutrition Animal Ethics Committees. The rabbits were housed individually in surroundings of controlled temperature (20 ± 1 °C) and a 12 h light cycle (06:00 h to 18:00 h).

All rabbits were initially fed a diet containing 0.25% (w/w) cholesterol that was mixed with their basic rabbit chow (IMVS, Gillies Plains, SA). This diet was fed to the rabbits for a period of 2 weeks to increase their plasma cholesterol concentrations prior to the administration of the crude catechin extract.

The rabbits were then randomised into four different treatment groups and the crude catechin extract was fed at concentrations of 0, 0.5, 1 or 2% (w/w). The extract was mixed in with their normal rabbit chow along with 0.25% (w/w) cholesterol and fed to the rabbits for a period of 28 days. Daily consumption of the diets was determined. Rabbits were fasted overnight and blood samples for lipid analysis were taken from the ear artery prior to and after the two-week cholesterol-only feeding period. Following the 4 weeks of dietary intervention with the crude catechin extract, the rabbits were fasted overnight and the following morning were injected intramuscularly (i.m.) with 1.2 ml (2.5 mg) of Acepromazine. Once sedated, rabbits were injected i.m. with a muscle relaxant (0.75 ml Rompun, 15 mg) and a general anaesthetic (1.5 ml Ketamine, 150 mg). Under deep anaesthesia the rabbits were bled by cardiac puncture until euthanasia. Blood was collected into EDTA tubes (1 mM) and plasma was isolated by centrifugation at $3000 \times g$ for 10 min at 4 °C. The entire aorta, from the ascending arch to the ileac bifurcation, was carefully removed and divided into three segments: the aortic arch, the descending aorta and the abdominal aorta. The aortic arch and the abdominal aorta were fixed and stained for atheroma assessment then quantified using