Green Tea Upregulates the Low-Density Lipoprotein Receptor through the Sterol-Regulated Element Binding Protein in HepG2 Liver Cells

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Green tea from *Camellia sinensis* lowers plasma cholesterol in animal models of hypercholesterolemia. The aim of this study was to determine the effects of green tea on the expression of the hepatic low-density lipoprotein (LDL) receptor, a cell surface protein involved in the control of plasma cholesterol. Incubating human HepG2 liver cells in culture with green tea increased both LDL receptor binding activity and protein. An ethyl acetate extract of green tea, containing 70% (w/w) catechins, also increased the LDL receptor binding activity, protein, and mRNA, indicating that (1) the effect was at the level of gene transcription and that (2) the catechins were the active constituents. The mechanism by which green tea up-regulated the LDL receptor was then investigated. Green tea decreased the cell cholesterol concentration (−30%) and increased the conversion of the sterol-regulated element binding protein (SREBP-1) from the inactive precursor form to the active transcription-factor form. Consistent with this, the mRNA of 3-hydroxy-3-methylglutaryl coenzyme A reductase, the rate-limiting enzyme in cholesterol synthesis, was also increased by green tea. In conclusion, green tea up-regulated the LDL receptor in HepG2 cells. The effect was most likely mediated through SREBP-1 in response to a decrease in the intracellular cholesterol concentration. The LDL receptor may therefore play a role in the hypocholesterolemic effect of green tea in vivo.

Keywords: *Green tea; LDL receptor; HepG2 cells; catechins; cholesterol; SREBP-1; HMG CoA reductase*

INTRODUCTION

Elevated plasma cholesterol is a major risk factor for the development of heart disease. Epidemiological studies in Japan have identified an inverse association between the consumption of green tea and the plasma concentration of cholesterol (*1*−*3*), a major risk factor in the development of heart disease (*4*). Consistent with this observation, numerous intervention studies in animal models have found that green tea (*Camellia sinensis*) or green tea extracts enriched in catechins exhibits a hypocholesterolaemic effect (*5*−*10*). The purified tea catechins, (−)-epicatechin gallate and (−)-epigallocatechin gallate, have also been observed to lower plasma cholesterol in mice (*6*) and rat (*11*) models of dietary-induced hypercholesterolemia. Therefore, the catechins, which account for more than one-third of the solids extracted in a normal tea infusion (*12*, *13*), appear to be the active components of green tea.

The mechanism of action for the lowering of plasma cholesterol by green tea may involve an enhanced clearance of cholesterol from the plasma as observed in rats fed (−)-epigallocatechin gallate (*11*), the most abundant of the catechins found in the tea (*12*, *13*). Such an increased clearance would most likely be the result of an increased expression of the low-density lipoprotein (LDL) receptor, the major mechanism by which cholesterol is removed from the circulation (*14*). However, the effect of green tea on the LDL receptor has yet to be investigated.

The aim of the present study was therefore to determine the effects of green tea on the expression of the LDL receptor. For this purpose, human HepG2 liver cells, known to express LDL receptors amenable to regulation (*15*), were cultured in the presence of increasing amounts of green tea. The tea was found to increase LDL receptor binding activity, protein, and mRNA. The mechanism by which green tea up-regulated the LDL receptor was then investigated. Several green tea extracts were also prepared by a series of solvent extractions and tested for their ability to up-regulate the receptor.

MATERIALS AND METHODS

Green Tea. The green tea used in this study was commercially available "Special Gunpowder" packaged by the China National Native Products and Animal By-products Import and Export Corp., Zhejiang Tea Branch, China. The green tea was prepared fresh for every experiment by brewing 10 g of green tea leaves for 10 min in 100 mL of boiled water (10% w/v) followed by paper filtration. Different solvent extracts of the green tea were also prepared and freeze-dried (*5*, *16*) as described in Figure 1.

HepG2 Cell Cultures. The HepG2 cells were grown in monolayer cultures to near confluency in 75 cm^2 flasks with 10 mL of Dulbecco's modified Eagle's media (DMEM) containing 10% (v/v) fetal calf serum (DMEM/FCS) at 37 °C with 5%

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10.1021/jf010275d CCC: $20.00
Published on Web 10/05/2001

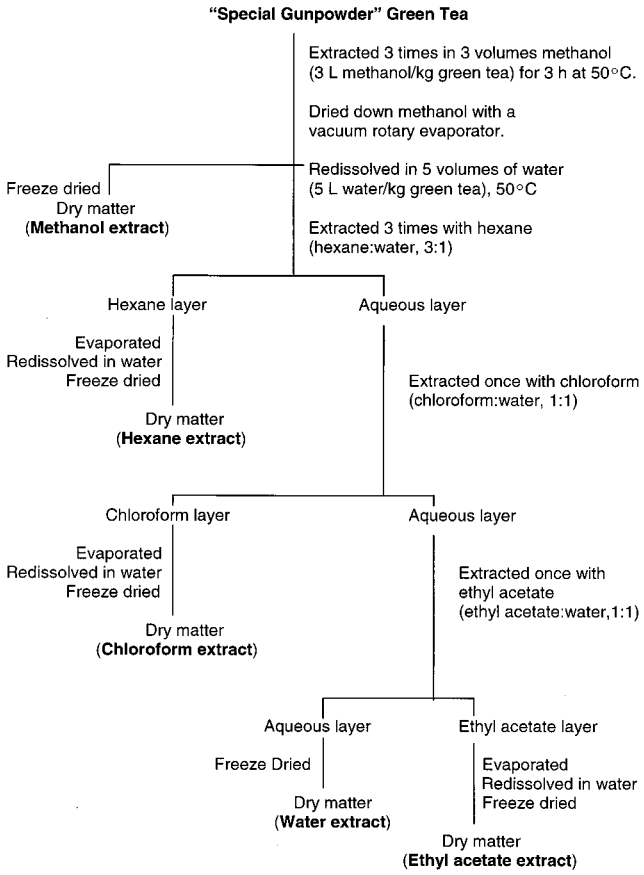

Figure 1. Flowchart for solvent extractions. Green tea was subjected to a series of solvent extractions as indicated. Five extracts were prepared: (1) methanol, (2) hexane, (3) chloroform, (4) water, and (5) ethyl acetate extracts.

CO_2 (*15*, *17*). The cells were then incubated for 24 h in 75 cm^2 flasks with 10 mL of DMEM/FCS containing different amounts (0−200 μL) of the 10% (w/v) freshly brewed green tea or different amounts of the solvent extracts—50 or 100 μM equivalence of (−)-epigallocatechin gallate. Three flasks of cells were treated with each dose of green tea or extract.

The amounts of the ethyl acetate dry matter extract (w/v) used in the HepG2 incubations were calculated to give (−)-epigallocatechin gallate concentrations equivalent to 50 or 100 μM on the basis of this catechin accounting for 60% of the catechins and all catechins accounting for 70% of the dry matter in an ethyl acetate extract (*5*). The other extracts were tested at the concentration (w/v) that was calculated to give 100 μM (−)-epigallocatechin gallate equivalence with the ethyl acetate extract.

LDL Receptor Binding Activity. Following incubation, the cells from each flask were harvested and resuspended in phosphate-buffered saline (PBS), and the protein content was measured (*18*). Determination of the specific LDL receptor binding activity was measured according to the method of Roach et al. (*19*). The intact HepG2 cells (100 μg of protein) were incubated for 1 h at room temperature with LDL-gold conjugates (20 μg of protein/mL) in the absence or presence of 20 mM EDTA to determine total and nonspecific binding, respectively. After 1 h, the cells were pelleted by centrifugation, washed, and resuspended in 4% (w/v) gum arabic, and a silver enhancement IntenSE BL kit solution (Amersham, Sydney, Australia) was then added. The silver enhancement reaction and absorbance measurements (500 nm) were carried out using the Cobas Bio autoanalyzer (Roche Diagnostica, Nutley, NJ). The amount of LDL bound to the cells was expressed as nanograms of LDL protein bound per milligram of cell protein (ng of LDL/mg of cell protein). Duplicate determinations were made for both total (−EDTA) and nonspecific (+EDTA) binding, and the specific binding (total binding minus nonspecific binding) was taken to be the measure of LDL receptor binding activity.

LDL Receptor Protein. Determination of LDL receptor protein was done by western blotting with a polyclonal anti-LDL receptor antibody (*20*). After incubation with the green tea, the HepG2 cells were frozen at −80 °C for at least 24 h and slowly thawed for analysis. Thawed cells were pelleted by centrifugation at 400*g* for 10 min and resuspended in solubilization buffer (250 mM Tris-maleate (pH 6.0), 2 mM $CaCl_2$, 1 mM phenylmethanesulfonyl fluoride, and 1 mM *N*-ethylmaleimide) to make a total volume of 300 μL in 1.5 mL plastic centrifuge tubes. To this was added 90 μL of 5% (w/v) Triton X-100, the mixture was vortexed, and the unsolubilized cell matter was removed by centrifugation at 400*g* for 15 min in a swinging bucket centrifuge. The protein content of the solubilized cell supernatant was then measured (*18*), and the samples (100 μg of protein) were subjected to electrophoresis on 3−15% sodium dodecyl sulfate−polyacrylamide gradient gels and electrotransferred onto nitrocellulose filter membranes. The membranes were then overlaid with the polyclonal antibody against the LDL receptor (*20*) followed by an anti-rabbit IgG antibody conjugated to horseradish peroxidase (Sigma, St. Louis, MO). The LDL receptor band was then detected on Hyperfilm-ECL X-ray film (Amersham, North Ryde, NSW, Australia) using an enhanced chemiluminescence (ECL) enzyme substrate kit (Amersham). Quantification of the LDL receptor protein on film was performed using an LKB Ultroscan XL enhanced laser densitometer and Gelscan computer program (Pharmacia LKB Biotechnology, North Ryde, NSW, Australia). Results are expressed as peak area in arbitrary absorbance units as determined from the densitometer scans.

LDL Receptor and HMG-CoA Reductase mRNA. To assay for the LDL receptor mRNA, total cellular RNA was first isolated using the procedure of Chomocznski and Sacchi (*21*). The LDL receptor mRNA was then measured using reverse transcription and the Polymerase Chain Reaction (PCR) to incorporate a nucleotide conjugated with digoxygenin into an amplified LDL receptor sequence (*22*).

The isolated HepG2 cell total RNA was reverse transcribed into cDNA along with an internal standard, AW109 (Perkin-Elmer Cetus Instruments, Norwalk, CT), a synthetic piece of cRNA which contains primer site sequences unique to the LDL receptor, using the Moloney murine leukemia virus reverse transcriptase (50 units/mL, Perkin-Elmer Cetus Instruments). The transcription was done by sequentially incubating the transcription mixture at 23 °C for 10 min, at 45 °C for 15 min, and at 95 °C for 5 min in a thermal cycler (Perkin-Elmer Cetus Instruments) and finally chilling on ice.

An LDL receptor sequence was then amplified using the PCR with AW125 and AW126 (Perkin-Elmer Cetus) as the downstream and upstream primers, respectively. During the amplification a modified nucleotide, dUTP conjugated to digoxygenin, was incorporated into the LDL receptor sequence. The amplification was done with a DNA thermal cycler (Perkin-Elmer Cetus) for 27 cycles of denaturation at 95 °C for 1 min, primer annealing at 55 °C for 1 min, and extension at 72 °C for 1 min. After 27 cycles, a further extension period of 10 min at 72 °C was done.

The amplified sequences were size fractionated by electrophoresis for 90 min at 90 V in 3% (w/v) agarose gels with 0.8 mM Tris acetate (pH 8.5) and 40 μM EDTA as running buffer, and the DNA was transferred onto positively charged nylon membranes (Boehringer Mannheim, Rose Park, Australia) by blotting for 4 h in 0.15 M trisodium citrate (pH 7.6) and 1.5 M NaCl. The sequences were then visualized by exposing the nylon membranes to hyper-film ECL (Amersham) after incubation with an anti-digoxygenin-IgG antibody conjugated to alkaline phosphatase (Boehringer Mannheim) and an ECL alkaline phosphatase substrate solution containing disodium 3-(4-methoxyspiro{1,2-dioxetane-3,2-(5-chloro)tricyclo-[3.3.1.1] decan}-4-yl)phenyl phosphate (AMPPD) (Boehringer Mannheima).

The films were then scanned using the LKB Ultrascan XL enhanced laser densitometer (Pharmacia LKB Biotechnology)

to determine the intensity of the two bands corresponding to (1) cellular LDL receptor mRNA at 258 bp and (2) synthetic AW109 internal standard RNA at 301 bp. The amount of LDL receptor mRNA in the HepG2 cells was calculated relative to the intensity of the band for the known amount of AW109 RNA added as internal standard and was expressed as copies per milligram of cellular RNA originally reverse transcribed.

The 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase mRNA of the HepG2 cells was measured using reverse transcription and the PCR, as described above for the LDL receptor mRNA. The AW109 cRNA was also used as the internal standard in this assay because it contains coding sequences of the HMG-CoA reductase gene. The same PCR reaction mixture was used except that HMG-CoA reductase-specific primers were included, namely, AW102 and AW104 (Perkin-Elmer Cetus) as downstream and upstream primers, respectively.

Sterol-Regulated Element Binding Protein (SREBP-1). Five 75 cm^2 flasks/treatment group were harvested, and the cells were pelleted by centrifugation at 500*g* for 10 min. The cells were then fractionated as described by Kawabe et al. (*23*). Aliquots (150 μg) of the resulting nuclear and membrane fractions were added to 2 times (v/v) loading buffer and boiled for 3 min (*24*). Once cooled, samples were run on 8% SDS-PAGE gels and transferred at 20 V overnight onto nitrocellulose membranes. The membranes were overlaid with a polyclonal antibody against the SREBP-1 protein (K-10, 1:200 dilution, Santa Cruz Biotechnology, Santa Cruz, CA) followed by a secondary antibody conjugated to horseradish peroxidase (1:5000 dilution, Sigma, St. Louis, MO). The inactive precursor and active transcription factor forms of SREBP-1 were then detected on X-ray film (Hyperfilm-ECL, Amersham) using ECL (SuperSignal Substrate, Rockford IL).

Cholesterol, Lathosterol, and Chenodeoxycholic Acid. Cells were frozen at −80 °C for at least 24 h and slowly thawed for analysis. Thawed cells were pelleted by centrifugation for 5 min at 400*g*. They were then homogenized by resuspending in 1 mL of SDS buffer (0.1% SDS, 1 mM EDTA, and 0.1 M Tris Base, pH 7.4) and taken up 6 times in an 18 gauge syringe; the protein content was then determined (*18*).

Total cholesterol (esterified plus free) and lathosterol, as an index of cholesterol synthesis (*25*), were measured by gas−liquid chromatography as described by Wolthers et al. (*26*). To measure unesterified cholesterol, the saponification step was omitted. The cholesterol and lathosterol concentrations were expressed relative to the amount of cell protein (milligrams per milligram of cell protein and micrograms per milligram of cell protein, respectively).

The cholesterol and chenodeoxycholic acid concentrations in the media were also determined. For cholesterol, 10 mL of the medium was reduced to near dryness using a Savant SpeedVac SC100 (Selby Anax, Adelaide, Australia), resuspended in 1 mL of water and analyzed as for the cells (*26*). For chenodeoxycholic acid, the cells were grown to near confluency in 75 cm^2 flasks as described above except that the green tea incubations (24 h) were done in 10 mL of DMEM medium free of phenol red (*27*). After the 24 h incubation, the media from two flasks were combined, and the bile acids were extracted from the 20 mL of medium by passage through a reversed phase C18 cartridge (Waters Associates, Milford, MA). Washes were done with 10 mL of water and 5 mL of 10% (v/v) methanol, and the bile acids were eluted with 10 mL of 85% (v/v) methanol (*27*). The samples were dried under a stream of nitrogen, and the bile acids were then methylated, derivatized with trifluoroacetic anhydride, and analyzed by gas−liquid chromatography using lithocholic acid as the internal standard (*28*). Due to the low concentration of bile acids in the medium of the HepG2 cells, only chenodeoxycholic acid was successfully detected.

Statistics. Results are expressed as mean ± SEM. Statistical evaluation was done using the Student *t* test, comparing treatment groups with the control. A value of $p < 0.05$ was taken as the criterion of significance.

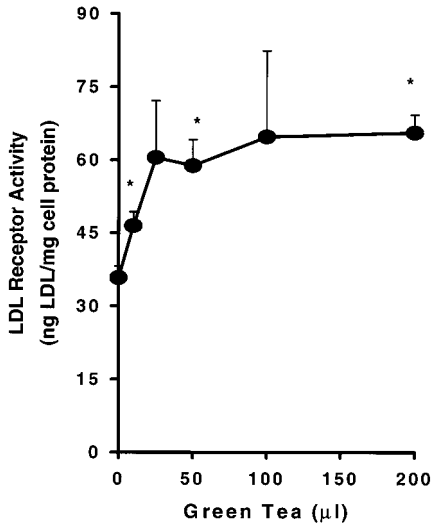

Figure 2. Dose-dependent effect of freshly brewed green tea on the LDL receptor binding activity. HepG2 cells were incubated for 24 h with increasing amounts of freshly brewed green tea, 0−200 μL in 10 mL of media. The LDL receptor binding activity was measured as the calcium-dependent binding of LDL-gold to the intact cells. The values are means ± SEM of triplicate cell incubations. The asterisk (*) denotes a significant difference compared to control ($p < 0.05$).

RESULTS

LDL Receptor. The addition of increasing amounts of freshly brewed green tea to the medium of the HepG2 cells increased the LDL receptor binding activity in a dose-dependent and saturable fashion (Figure 2). The amount of LDL-gold binding to the intact HepG2 cells was significantly greater than control even with the addition of only 10 μL of tea to 10 mL of medium and attained a plateau from 25 μL onward. The green tea also caused an increase in the amount of LDL receptor protein detected by blotting with the polyclonal LDL receptor antibody. The scanned peak area was increased almost 3-fold (5.76 ± 1.36 arbitrary absorbance units) in cells incubated in the presence of 200 μL of green tea compared to control cells (1.90 ± 0.32 units). The increase in LDL receptor binding activity was therefore most likely due to a marked increase in the number of LDL receptors in the cells with some available to bind LDL on the outer surface of the HepG2 cells.

The catechins, present in high amounts in green tea, have been proposed to be the constituents that lower plasma cholesterol in animal models (*5*, *6*, *11*). Extracts of the green tea were therefore prepared as shown in Figure 1 and tested for their ability to up-regulate the LDL receptor of the HepG2 cells. As seen in Figure 3, the preparations from methanol, which efficiently extracts the catechins from the green tea leaves, and ethyl acetate, which effectively purifies polyphenolic compounds such as the catechins, caused marked increases in the LDL receptor binding activity of the HepG2 cells. On a dry weight basis, these extracts were also more effective at up-regulating the LDL receptor than the freshly brewed green tea used as a positive control. This may have been due to a higher relative content of catechins in these extracts.

In contrast, the preparations from hexane and chloroform, which extract pigments, caffeine, and oily substances, had no effect (Figure 3). There was also no significant activity left in the water phase after extraction with ethyl acetate. The catechins, which account

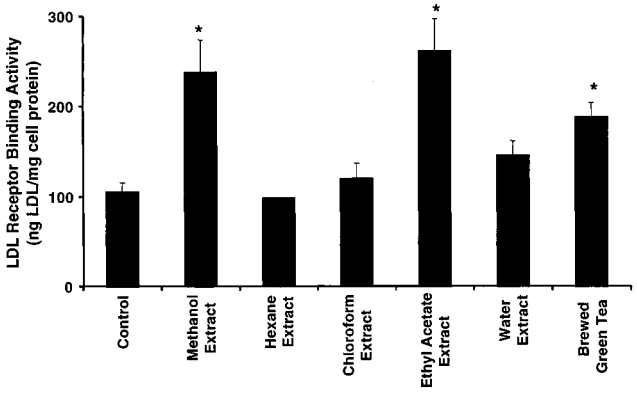

Figure 3. Effect of different green tea extracts on LDL receptor binding activity. HepG2 cells were incubated for 24 h with the indicated solvent extracts of green tea as prepared according to the procedure illustrated in Figure 1. The concentration (w/v) of the ethyl acetate extract used in the media was calculated to represent 100 μM epigallocatechin gallate equivalence as described under Materials and Methods. For direct comparison, the same concentration (w/v) of the other extracts was used. Freshly brewed green tea (100 μL in 10 mL of medium) was used as a positive control. Values are means ± SEM of triplicate cell incubations. The asterisk (*) denotes a significant difference compared to control ($p < 0.05$).

Table 1. Effect of an Ethyl Acetate Extract from Green Tea on the LDL Receptor Binding Activity, Protein, mRNA, and HMG-CoA Reductase mRNA of HepG2 Cells[a]

	(−)-epigallocatechin gallate equivalence (μM)		
	0	50	100
LDL receptor activity (ng of LDL/mg of cell protein)	34 ± 4	57 ± 3	140 ± 5
LDL receptor protein (arbitrary absorbance units)	0.3 ± 0.1	3.0 ± 0.2	6.6 ± 0.4
LDL receptor mRNA (copies × 10^5/μg of cell RNA)	3.5 ± 0.2	70.0 ± 3.6	79.3 ± 3.6
HMG-CoA reductase mRNA (copies × 10^5/μg of cell RNA)	4.0 ± 0.3	26.6 ± 2.8	57.4 ± 4.0

[a] Values are mean ± SEM of triplicate cell incubations and are all significantly different from each other ($p < 0.05$) for each parameter measured.

for up to 70% of the dry matter extracted by ethyl acetate, may therefore be the constituents of green tea that up-regulate the LDL receptor.

As well as increasing the LDL receptor binding activity, the ethyl acetate extract increased the amount of LDL receptor protein in the HepG2 cells 9- and 21-fold (Table 1) and the LDL receptor mRNA 21- and 24-fold (Table 1) at 50 and 100 μM (−)-epigallocatechin gallate equivalence, respectively. The green tea extract therefore most likely increased the LDL receptor at the level of gene transcription.

The ethyl acetate extract also increased the amount of HMG-CoA reductase mRNA in the HepG2 cells 6- and 14-fold at 50 and 100 μM (−)-epigallocatechin gallate equivalence, respectively (Table 1).

Cell Cholesterol. In an attempt to determine how green tea up-regulated the LDL receptor, the HepG2 cell cholesterol concentration was measured; a deficiency in intracellular cholesterol is well-known to up-regulate the LDL receptor (*29*). Treatment of the cells with freshly brewed green tea significantly decreased the intracellular total cholesterol concentration (esterified plus unesterified cholesterol) at each of the doses tested, with most of the decrease occurring (−30%) at the lowest dose tested—50 μL in 10 mL of medium (Figure 4).

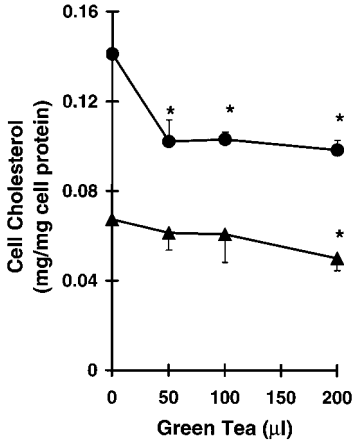

Figure 4. Dose-dependent effect of freshly brewed green tea on intracellular total and free cholesterol concentrations. HepG2 cells were incubated for 24 h with increasing amounts of green tea, 0−200 μL in 10 mL of medium. Homogenized cells were extracted with hexane and total cholesterol (●) and free cholesterol (▲) were analyzed using gas−liquid chromatography and expressed relative to cell protein. The values are means ± SEM of triplicate cell incubations. The asterisk (*) denotes a significant difference compared to control ($p < 0.05$).

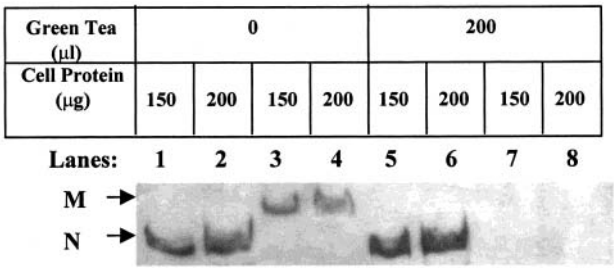

Figure 5. Effect of green tea on SREBP. Cells were fractionated for nuclear and membrane fractions following incubation in the presence of freshly brewed green tea. Cellular proteins were separated and identified using SDS-PAGE and Western blotting (see Materials and Methods). The inactive precursor (membrane form; M) and the active transcription factor form (nuclear cell fraction; N) of SREBP-1 were then detected on X-ray film using ECL. Lanes 1−4 represent cells that have not been exposed to green tea. Lanes 5−8 represent cells exposed to green tea.

Green tea also decreased the intracellular concentration of unesterified cholesterol, but this effect was significant only at the highest dose of 200 μL (Figure 4). Green tea may therefore have reduced the intracellular concentration of cholesterol and thereby triggered an increase in the expression of the LDL receptor.

SREBP-1. Sterol feedback regulation is mediated through transcription factors (SREBPs) that bind to the sterol regulatory element of the LDL receptor gene (*14*, *23*, *24*). The effect of the green tea induced low cellular cholesterol content on SREBP-1 was therefore studied. Treatment with green tea (200 μL) resulted in 62% (150 μg of cell protein) and 65% (200 μg of cell protein) increases in the active transcription factor form of SREBP-1 (nuclear cell fraction {N}, lanes 5 and 6), compared to the respective control (lanes 1 and 2) (Figure 5). In addition to this, green tea treatment decreased the inactive precursor form of SREBP-1 (membrane fraction; M) to undetectable levels (lanes 7 and 8). This increase in the conversion of SREBP-1, from its inactive precursor form to its active transcription factor form, may therefore have led to an increase in the transcription of the LDL receptor gene.

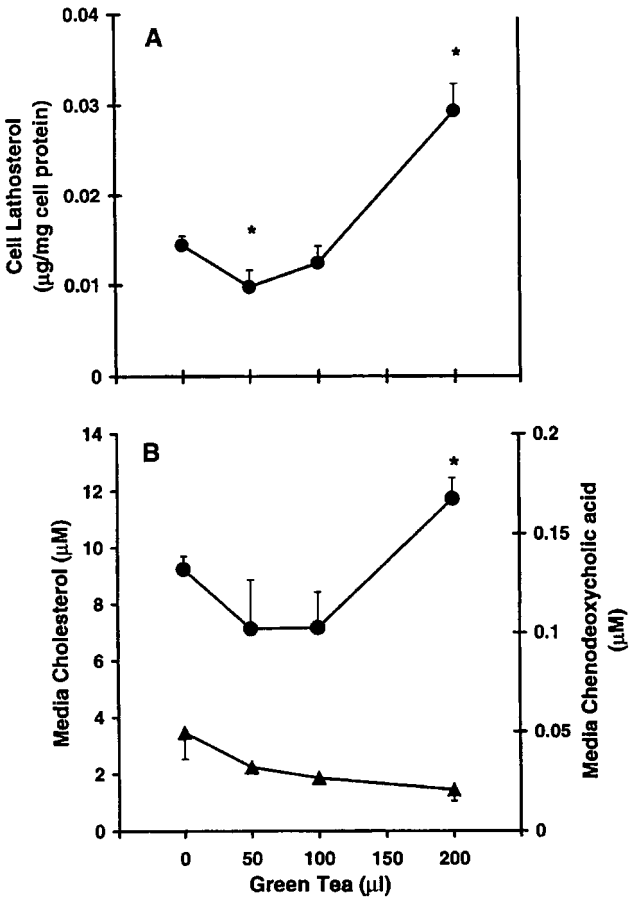

Figure 6. Dose-dependent effect of freshly brewed green tea on cholesterol synthesis. HepG2 cells were incubated for 24 h with increasing amounts of green tea, 0−200 μL in 10 mL of medium. Lathosterol (A, ●) was extracted from homogenized cells and measured using gas chromatography. Media cholesterol (B, ●) and chenodeoxycholic acid (B, ▲) were also determined using gas chromatography. Values are means ± SEM of triplicate cell incubations. The asterisk (*) denotes a significant difference compared to control ($p < 0.05$).

Cholesterol Synthesis, Medium Cholesterol, and Chenodeoxycholate. A reduction in cholesterol synthesis, an increase in the conversion of cholesterol into bile acids, and a loss of cholesterol from the cell into the medium are three factors that could have decreased the intracellular concentration of cholesterol in the HepG2 cells (*15*, *27*). Indices of these cholesterol homeostatic mechanisms were therefore measured.

The cellular lathosterol concentration, measured as an index of cholesterol synthesis (*25*, *26*), revealed that green tea significantly reduced cholesterol synthesis at the lowest dose of 50 μL (Figure 6A). At this concentration, green tea may therefore have lowered the intracellular concentration of cholesterol by inhibiting de novo cholesterol synthesis. However, there was no significant difference from control at the higher dose of 100 μL, and at 200 μL there was a significant 2-fold increase in the cellular lathosterol concentration (Figure 6A). This biphasic effect on the cholesterol synthesis index indicates that different mechanisms of action may have operated depending on the concentration of the green tea.

The green tea did not produce any significant changes in the concentration of chenodeoxycholic acid in the media (Figure 6B). The lowered intracellular cholesterol concentration (Figure 4) was therefore not likely to be due to an increase in the conversion of cholesterol to bile acids.

At the lower doses of 50 and 100 μL in 10 mL of media, the green tea tended to lower the cholesterol concentration in the media, but this did not reach significance (Figure 6B). At the highest dose of 200 μL, however, green tea caused a significant increase in the media cholesterol concentration. The concentration was 25% above that measured in the media of control cells (Figure 6B). There therefore appeared to be an increase in the export of cholesterol from the cells into the media when the cells were exposed to the 200 μL dose, as the intracellular cholesterol remained significantly decreased (Figure 4) despite the apparent increase in cholesterol synthesis at this dose (Figure 6A). The increased cellular cholesterol synthesis appeared to be the source of the extra medium cholesterol because there was a very high correlation ($r = 0.956$, $p < 0.01$) between the cell lathosterol concentration (Figure 6A) and the medium cholesterol concentration (Figure 6B).

DISCUSSION

The aim of the present study was to determine the effects of green tea on the expression of the LDL receptor, a cell surface protein that is involved in the control of plasma cholesterol (*29*). When human HepG2 liver cells were cultured in the presence of freshly brewed green tea, LDL receptor binding activity and protein were significantly increased compared to control. An ethyl acetate extract of the green tea also increased the LDL receptor binding activity, protein, and mRNA. This indicated that the tea catechins, the major constituents extracted by ethyl acetate (*5*), were likely to be the active constituents. This also indicated that the effect was likely to be at the level of gene transcription—the level at which the LDL receptor is known to be regulated (*14*, *29*).

Cholesterol is known to regulate hepatic LDL receptor gene expression through the activation and deactivation of transcription factors called SREBPs (*14*). When the cholesterol content of the cell falls, SREBPs, which reside in an inactive form in the cytoplasm, are cleaved by proteases and are activated as transcription factors. The active SREBPs then migrate to the nucleus of the cell and bind to the LDL receptor gene and stimulate it to produce more LDL receptor protein, thereby allowing more cholesterol to enter the cell. We hypothesized that the catechins in green tea induce the LDL receptor through regulation of SREBPs. The results demonstrated that when the hepatocytes were incubated in the presence of freshly brewed green tea, the cell cholesterol content was 30% lower and the conversion of SREBP-1 from its inactive precursor form to its active transcription form was increased. This may therefore have triggered the up-regulation of the LDL receptor and also the observed increase in the HMG-CoA reductase mRNA, as both genes are turned on by a deficiency in cell cholesterol (*29*) through SREBPs as their common sterol feedback mechanism (*14*).

At the lowest dose of green tea (50 μL in 10 mL of medium) there appeared to be a decrease in cholesterol synthesis measured as cell lathosterol, a cholesterol precursor used as an index of cholesterol synthesis (*25*, *26*). At this concentration, green tea may have inhibited cholesterol synthesis which, in turn, may have contributed to the reduction in cell cholesterol. In contrast, cell

lathosterol was increased 2-fold over control at the highest dose of green tea (200 μL in 10 mL of medium). However, despite this apparent increase in cholesterol synthesis, cholesterol did not accumulate within the cells as the cellular cholesterol remained decreased by >30%. Instead of accumulating in the cells, the extra cholesterol was found in the medium where its concentration was increased by 25% over control. Therefore, at the high concentration, green tea appeared to increase the export of cholesterol from the cells into the media to such an extent that the increase in cholesterol synthesis and the LDL receptor did not fully compensate for the loss of cell sterol. Interestingly, there was a very high correlation ($r = 0.956$) between cell lathosterol and the concentration of cholesterol in the media over all of the green tea concentrations. This suggests that the concentration of cholesterol in the media was directly linked to the amount of cholesterol synthesized by the cells. An increase in the conversion of cell cholesterol into bile acids (*27*) did not appear to be a factor in the reduction of cell cholesterol as the chenodeoxycholic acid in the media tended to go down rather than up.

Despite its up-regulation, the LDL receptor did not affect the media cholesterol concentration in that there was no reciprocal relationship evident between the medium cholesterol concentration and the LDL receptor activity. This may be difficult to achieve given that (1) equilibration of cholesterol occurs through passive diffusion and (2) cells secrete cholesterol-carrying lipoproteins that may or may not be able to be taken up through the LDL receptor system. Changing the amount of cholesterol in the media certainly has been shown to result in changes in cholesterol content within the cells and thereby in changes in the LDL receptor expression, but the reverse has not (*14*, *29*).

Nonetheless, if the green tea catechins have the same effects on the liver in vivo, the increased hepatic LDL receptor expression could explain the enhanced clearance of cholesterol from the plasma seen in rats fed (−)-epigallocatechin gallate, the major tea catechin (*11*). In animal models, liver cells may also be deprived of cholesterol because the tea catechins decrease the intestinal absorption of cholesterol and cause an increased excretion of neutral sterols in the feces (*5*, *10*, *11*). This appears to be because the catechins, (−)-epigallocatechin gallate in particular, can form an insoluble complex with cholesterol and thereby reduce the solubility of cholesterol in bile acid micelles (*30*).

The ability of the catechins to form complexes with cholesterol may also explain the effects seen in vitro with the highest dose of green tea in the present study. At this concentration the catechins may complex with enough cholesterol in the media to render the media essentially cholesterol-deficient. Cholesterol would then move from the cells into the media by normal diffusion down the concentration gradient. This could explain why the concentration of cholesterol is seen to increase in the media, whereas the cells are not able to regain their normal intracellular cholesterol levels despite an increase in cholesterol synthesis. Two other possibilities that could explain the accumulation of cholesterol in the media are as follows: (1) green tea may block the binding of lipoproteins to the LDL receptor so they are unable to be endocytosed or (2) the types of lipoproteins secreted by the cells in the presence of green tea are not able to be taken up by the cells through the LDL receptor.

So far, intervention studies with green tea in humans have not resulted in significant cholesterol lowering (*31*, *32*). However, the studies were mainly designed to study the antioxidant potential of green tea and were conducted in normocholesterolemic subjects. Studies in diet-sensitive hypercholesterolemic subjects are therefore needed to truly test the cholesterol-lowering potential of green tea in humans. The amount of green tea catechins ingested is also likely to be of importance. Interestingly, in one study (*32*), a polyphenol extract that gave 3 times the amount of catechins relative to the green tea dose resulted in an 8% reduction in plasma cholesterol compared to a 3% reduction with green tea. The effect was very close to significance ($p = 0.06$) in these normocholesterolemic subjects and may reach significance in more diet responsive hypercholesterolemic subjects.

At the concentrations of green tea tested in the present study (10−200 μL in 10 mL of media), we estimate the total catechin concentration to have ranged from 10 to 200 nM. These are therefore well within the concentrations that have been observed in plasma (>400 nM) after the ingestion of green tea (*33*).

In conclusion, green tea was observed to decrease the intracellular cholesterol concentration of HepG2 cells and up-regulated their LDL receptor. The effect on the receptor appeared to be at the level of gene transcription, and the catechins, the major constituents of green tea, appeared to be the active constituents. The effect was most likely mediated through SREBP-1 in response to a decrease in the intracellular cholesterol concentration. Up-regulation of the LDL receptor may therefore play a role in the hypocholesterolemic effect of green tea in vivo.

ABBREVIATIONS USED

DMEM, Dulbecco's modified Eagle's media; ECL, enhanced chemiluminescence; FCS, fetal calf serum; HepG2, human hepatoma cell line; HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; LDL, low-density lipoproteins; PCR, Polymerase Chain Reaction.

ACKNOWLEDGMENT

We thank Dr. Ian Record, CSIRO Health Sciences and Nutrition, for providing some of the ethyl acetate extract used in this study and Dr. Paul Kroon and Elizabeth Powell, University of Queensland Department of Biochemistry, for teaching us the LDL receptor mRNA assay. We also thank Dr. Mavis Abbey and Dr. Paul Nestel for their support of this work.

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Received for review March 2, 2001. Revised manuscript received July 26, 2001. Accepted July 26, 2001. C.B. and S.P. were the recipients of University of Adelaide scholarships.

JF010275D