Available online at www.sciencedirect.com
SCIENCE DIRECT®
Antiviral Research 58 (2003) 167–173
Antiviral Research
www.elsevier.com/locate/antiviral

Inhibition of adenovirus infection and adenain by green tea catechins

Joseph M. Weber*, Angelique Ruzindana-Umunyana, Lise Imbeault, Sucheta Sircar

Departement de Microbiologie et d'Infectiologie, Faculte de Medecine, Universite de Sherbrooke, Sherbrooke, Que., Canada J1H 5N4

Received 30 May 2002; accepted 22 November 2002

Abstract

Green tea catechins have been reported to inhibit proteases involved in cancer metastasis and infection by influenza virus and HIV. To date there are no effective anti-adenoviral therapies. Consequently, we studied the effect of green tea catechins, and particularly the predominant component, epigallocatechin-3-gallate (EGCG), on adenovirus infection and the viral protease adenain, in cell culture. Adding EGCG (100 μM) to the medium of infected cells reduced virus yield by two orders of magnitude, giving and IC_{50} of 25 μM and a therapeutic index of 22 in Hep2 cells. The agent was the most effective when added to the cells during the transition from the early to the late phase of viral infection suggesting that EGCG inhibits one or more late steps in virus infection. One of these steps appears to be virus assembly because the titer of infectious virus and the production of physical particles was much more affected than the synthesis of virus proteins. Another step might be the maturation cleavages carried out by adenain. Of the four catechins tested on adenain, EGCG was the most inhibitory with an IC_{50} of 109 μM, compared with an IC_{50} of 714 μM for PCMB, a standard cysteine protease inhibitor. EGCG and different green teas inactivated purified adenovirions with IC_{50} of 250 and 245-3095, respectively. We conclude that the anti-adenoviral activity of EGCG manifests itself through several mechanisms, both outside and inside the cell, but at effective drug concentrations well above that reported in the serum of green tea drinkers.

Keywords: Adenovirus type 2; Adenain; Protease; Green tea; EGCG; Catechins

1. Introduction

Adenoviruses are implicated in a variety of human and animal pathologies and are currently under intensive investigation as gene transfer vectors (Horwitz, 1996; Qualikene et al., 2000; Whickam, 2000; Doronin et al., 2001). Although absolved in the past of oncogenic potential in humans, because of a failure to find viral nucleic acids in tumors, evidence was recently reported supporting a possible "hit and run" mechanism (Nevels et al., 2001). Because of the large number of serotypes infecting man, little effort has been directed towards the development of antiviral strategies (De Clercq, 1993; Cornish et al., 1995; Sircar et al., 1996, 1998; Mentel et al., 1997, 2000; Mentel and Wegner, 2000; Ruzindana-Umunyana et al., 2000; Ruzindana-Umunyana and Weber, 2001; Pang et al., 2001). Based on our studies of the adenovirus protease, adenain, we have suggested this enzyme as a target for chemotherapy of adenovirus infections in general.

The human adenovirus 2 endopeptidase (adenain) is a 204 amino acid monomeric cysteine protease (reviewed in Weber, 1999). For optimal activity, adenain requires its eleven residue activating peptide, pH 8 and 45 °C in the presence of 1 mM thiol compounds and negatively charged polymers. The spatial disposition of the active site residues (H54, E/D71, C122) is identical to that of papain (H159, N175, C25) and so is the location of Q115 of adenain and Q19 of papain (Ding et al., 1996). In consequence some papain inhibitors also inhibit adenain (Sircar et al., 1998). The enzyme is specific for two consensus sites (M, I, L)XGG-X or (M, I, L)XGX-G, where X is any amino acid. A group of proteases with similar substrate specificity and related structures includes the cysteine proteases of African swine fever virus, vaccinia virus, fowlpox virus, and the Ubl-specific proteases of yeast and Chlamydia (Li and Hochstrasser, 1999; Lopez-Otin et al., 1989). Adenovirus maturation, infectivity and uncoating are dependent on proper adenain activity, suggesting that the viral protease is an appropriate target for the development of antivirals (Weber, 1999; Balakirev et al., 2002; Mangel et al., 2001; Pang et al., 2001).

There is an extensive literature documenting the anticancer activity of green tea catechins, particularly the

* Corresponding author. Tel.: +1-819-5645326; fax: +1-819-5645392.
E-mail address: joseph.weber@usherbrooke.ca (J.M. Weber).

0166-3542/02/$ – see front matter © 2002 Elsevier Science B.V. All rights reserved.
doi:10.1016/S0166-3542(02)00212-7

predominant component, epigallocatechin-3-gallate (EGCG; Jankun et al., 1997; Morre et al., 2000; Mukhtar and Ahmad, 1999; Cao and Cao, 1999; Yang et al., 1998b). The mechanism of inhibition has been attributed in part to the inhibition of certain proteases involved in metastasis (Garbisa et al., 1999, 2001; Demeule et al., 2000). In addition, EGCG has also been shown to have inhibitory effects on HIV and influenza virus (De Clercq, 2000; Haneda et al., 2000; Nakayama et al., 1993; Yamaguchi et al., 2002). Pharmacokinetic studies in humans showed that 77% of EGCG was present in plasma in the free form and that there was virtually no hepatic alteration or elimination as manifested by a half life of 5 h (Lee et al., 2002; Cai et al., 2002). The safety of the compound is attested to by a long history of green tea consumption by some asian populations. Here we report the effect of green tea catechins, and particularly that of EGCG, on adenovirus infection and adenain.

2. Materials and methods

2.1. Virus and cells

Hep2, is a human epidermoid carcinoma of the larynx, cell line. HMEC, is a normal human epithelioid cell line kindly provided by Dr. A. Jamali (originally from Clonetics Corp., Walkersville, MD; Guo et al., 2000). Both cell lines were cultured in DMEM medium and 10% fetal bovine serum. Human adenovirus type 2 (Ad2; originally obtained from R. Huebner, NIH) was grown in Hep2 cells and titered by either plaque formation or end-point dilution in Hep2 cells. All experiments were done at an m.o.i. of 10 PFU per cell and virus yield was titered by two-fold dilutions in 96 well microtiter plates as described (Precious and Russell, 1985). Labeling with ^{35}S-methionine, gel electrophoresis (SDS–PAGE) and virus purification methods were described before (Sircar et al., 1998).

2.2. Protease assays

Recombinant adenovirus type 2 protease (Adenain; EC3.4.22.39) was purified by chromatography from an *E. coli* expression system (pLPV) as described before (Keyvani-Amineh et al., 1995). Protease assays were done as described before with substrates of ts1 infected cell lysates labeled with ^{35}S-methionine at the non permissive temperature (39 °C) at 24 h p.i. as a source of viral precursor proteins, particularly pVII (Keyvani-Amineh et al., 1995). Ts1 is defective for protease activity at 39 °C, consequently providing a ready source of viral precursor proteins. This substrate was boiled to inactivate any residual adenain. Adenain was preincubated with inhibitors for 30 min. before substrate addition. Enzyme activities were assessed from the conversion of viral precursor protein pVII to VII as visualized on autoradiograms of SDS–PAGE separations. Recombinant human SCCA1 was purified from a yeast expression system (generously supplied by P. Pemberton).

2.3. MTT assay

The stock solution of MTT, 3-(4,5-dimethylthiozol-2-yl)-3,5-dipheryl tetrazolium bromide (Sigma cat. no. M2128) was prepared in sterilized phosphate-buffered saline at 2 mg/ml. Cells were plated in 96-well Linbro plates and 24 h later the medium was removed and replaced with fresh medium containing EGCG. The control wells contained an equivalent amount of solvent. Three days later the culture medium was carefully removed from the wells without disturbing the attached cells. MTT (10 μl in 200 μl of medium) was added to each well and incubated at 37 °C for 4 h. We found that 4 h was sufficient for the cells to reduce MTT to formazan crystals. The excess MTT was carefully removed and the formazan crystals were solubilized by adding 200 μl DMSO to each well and the absorbance measured at 550 nm.

2.4. Inhibitors

The green tea catechins epicatechin (EC), epicatechin-3-gallate (ECG), epigallocatechin (EGC) and epigallocatechin-3-gallate (EGCG) and the general cysteine protease inhibitor *p*-chloromercuribenzoate (PCMB) were purchased from Sigma–Aldrich. EGCG was also purchased from LKT Laboratories (2233 University Avenue, W., St. Paul, MN 55114-1629). The identity and purity of the molecules were verified by their absorbance spectrum and by mass spectrometry. Stock solutions were made as follows: catechins, 10 mM in phosphate buffered saline (PBS); PCMB, 20 mM in 10 mM NaOH diluted with bicarbonate buffer at pH 9.5. Green tea infusion was prepared by adding either 20 ml (designated as GT-20) or 100 ml (designated as GT-100) of boiling nanopure water to 1 gm of dry tea leaves for 10 min centrifuged and filtered through a 0.22 μm filter. EGCG concentrations were determined by spectroscopy using purified EGCG to construct the standard curve at a maximum absorbance of 278 nm. The efficacy of this preparation did not appear to change upon freezing and short-term storage (1 week at 4 °C). The different brands of tea were purchased from local supermarkets.

3. Results and discussion

3.1. Inhibition of virus replication

The anti-adenoviral activity of EGCG was evaluated in vitro in a time-course experiment. One hundred micromolar EGCG was added to infected cells at different times after infection and the virus yield was determined at 48 h as a percentage of the yield obtained in the absence of EGCG. The results are shown in Fig. 1. EGCG depressed virus yield by two orders of magnitude when it was added between 8 and

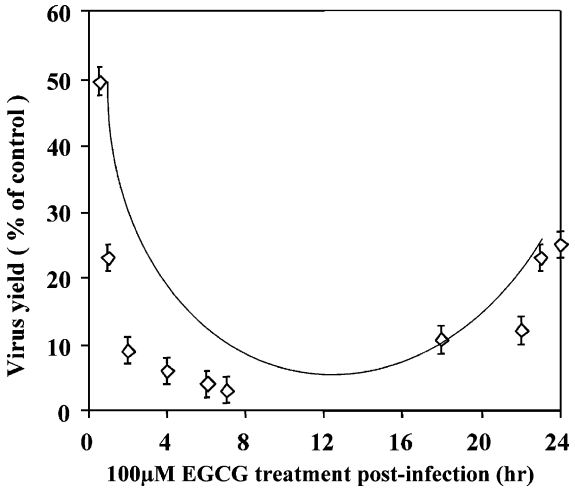

Fig. 1. Effect of EGCG on virus infection: time-course of treatment. One hundred micromolar EGCG was added to the medium of infected cells at different times after infection and at 48 h the virus yield was titered.

16 h after infection. The shape of the curve clearly suggests an inhibitory effect on the late phase of virus infection. Some aspect of the early phase however also appears to be drug-sensitive because treatment of the cells for 1 h prior to infection also showed a 50% decrease in subsequent virus yield (results not shown). Green tea infusion had a similar effect. This sensitivity could be mediated by the inhibition of some cellular process that virus infection relies upon. This time of addition study also suggests that the antiviral activity of the compound may be unstable after several hours.

The results of a dose–response experiment are shown in Fig. 2. Different concentrations of EGCG were added 18 h post-infection and the total virus (cells plus medium) or cell-associated virus yield was titered at 48 h. The divergence of the two curves suggests that EGCG not only inhibits virus synthesis but also promotes the release of the virus from the cells. This suggests an effect on the cell membrane by EGCG. Two reports have indeed suggested such an effect

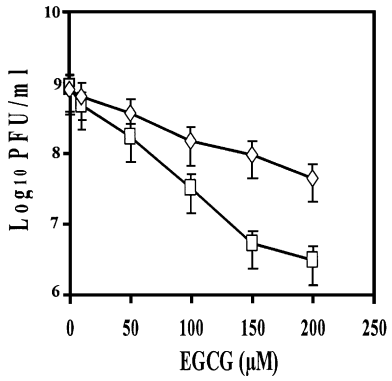

Fig. 2. Effect of EGCG on infectious virus production. EGCG was added to the medium of infected cells at 18 h post-infection and at 48 h the virus yield was titered from the cell pellet (cell-associated virus, □) and also from the combined medium plus cells (total virus, ◇).

Table 1
The antiviral effect of EGCG and green tea

Agent	IC_{50} (μM)	
	Inactivation of adenovirus	Effect on infectious virus production
Epigallocatechin gallate (EGCG)	250	25
Green tea infusions[a] (GT-20)		
Gunpowder	245	34
Uncle Lee's	2840	N.D.
Celestial seasonings	3095	45
Tetley	490	45

[a] Expressed in terms of estimated EGCG concentration in the tea.

(Chen et al., 1998; Morre et al., 2000). The IC_{50} for total virus yield was 25 μM (Table 1).

The virus particles were purified from a similar experiment and the proteins separated by SDS–PAGE (Fig. 3). At EGCG concentrations of 200 μM or greater, virus assembly was significantly reduced. This was in agreement with the titration experiments. It is of interest to note here that the assembled virions contained a normal set of polypeptides typical of mature virions (Fig. 3, lanes b–d).

Green tea infusions were also tested by adding 1 ml dilutions in DMEM to infected cells at 18 h post-infection for 15 min then 3 ml DMEM and incubated until 44 h post-infection and the virus yield titrated. The results from several types of teas, expressed in terms of EGCG concentration, were similar to those obtained using purified EGCG, as above (Table 1).

The observed antiviral effect of EGCG could be due to cellular toxicity. Green tea catechins, and particularly EGCG has been reported to be more toxic to cancer cells than normal cells (Sachinidis et al., 2000; Morre et al., 2000). As all of our experiments were conducted in Hep2 cells, which was originally derived from a human laryngeal epithelial carcinoma, we compared the toxicity of EGCG to Hep2 cells versus a normal epithelial cell line, HMEC. The MTT assay for toxicity over a 2-day period of treatment only showed a marginal difference between the two cell lines, neither showing any significant degree of impairement of cell survival (Fig. 4, panel A). The 50% cytotoxic concentration (CC_{50}) of EGCG to Hep2 cells was 540 μM. The antiviral effect of EGCG in the above experiments gave an IC_{50} of around 25 μM, which yields a therapeutic index (TI) of 22.

Toxicity was also assessed by a metabolic labeling experiment. Different concentrations of EGCG were added to the medium at 18 h after virus infection or mock-infection of Hep2 cells and then labeled with ^{35}S-methionine at 20–21 h. The cell lysates were separated by SDS–PAGE and autoradiographed. Neither cellular nor viral protein synthesis was affected significantly at 100 μM EGCG, but at 500 μM viral synthesis was depressed more than cellular (Fig. 4, panel B). These results also confirm the absence of toxicity of

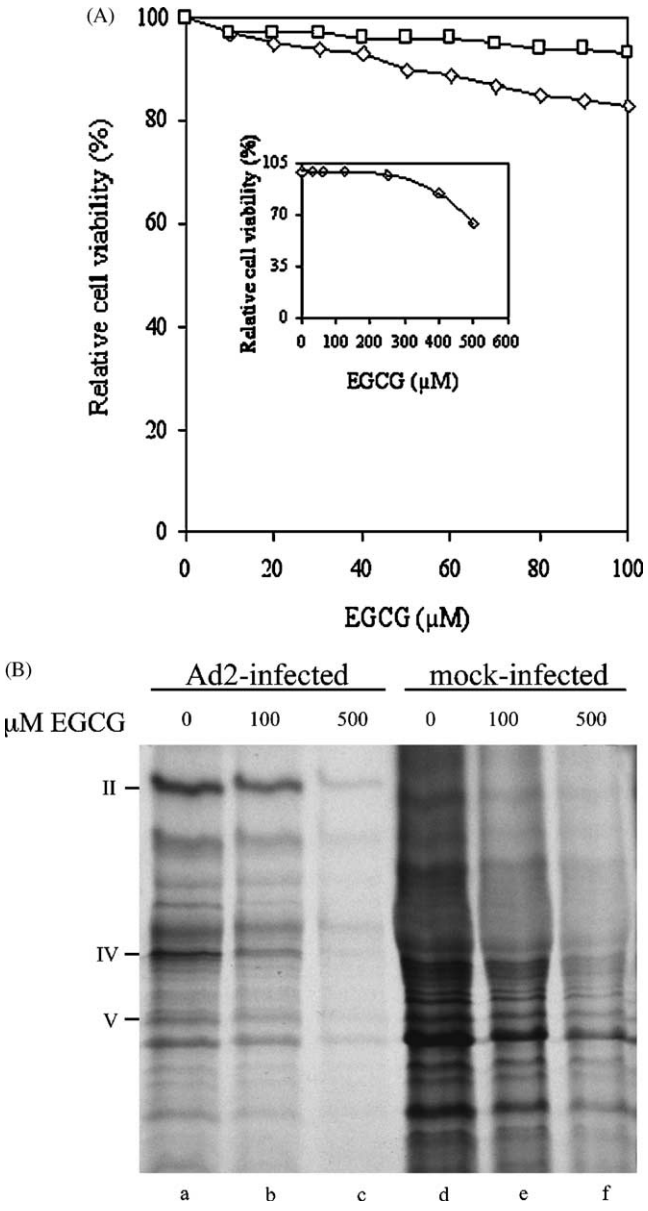

Fig. 3. EGCG toxicity. Panel A: effect on cell viability. Hep2 cells (cancer) and HMEC (normal) cells were grown in the presence of EGCG for 40 h and the relative cell viability was determined by the MTT test. The inset shows the effect of EGCG on Hep2 cells at higher concentrations. Panel B: effect on protein synthesis. Hep2 cells were Ad2-infected (a–c) or mock-infected (d–f) and EGCG was added to the medium at 18 h and ^{35}S-methionine at 20–21 h. The cells were lysed and subjected to SDS–PAGE and autoradiography.

EGCG at concentrations of up to, and somewhat beyond, 100 μM.

Taken together, the above experiments suggest that EGCG inhibits one or more late steps in virus infection. One of these steps appears to be virus assembly because the titer of infectious virus and the production of physical particles was much more affected than the synthesis of virus proteins. In agreement with published reports it is implicit from these results that catechins and EGCG in particular, are efficiently taken up by cells (Yang and Wang, 1993; Williams et al., 2000).

3.2. Inactivation of virions

We next tested the antiviral effect of EGCG in an extracellular environment. Highly concentrated purified virus was exposed to different concentrations of EGCG for 15 min at room temperature then dilutions made and the effect on infectivity titrated in Hep2 cells. We chose these conditions based on pilot experiments which suggested that variations in temperature and time of exposure had little effect. The direct antiviral effect of the first lot of EGCG from Sigma–Aldrich gave an IC_{50} of around 1 μM. The antiviral effect was not due to a delay in the infectious cycle because the virus titer did not increase by prolonging incubation. Three subsequent lots of EGCG from Sigma–Aldrich and one lot from LKT Laboratories, however, gave values of IC_{50} of only around 250 μM. The reason for this discrepancy in antiviral efficacy remains unknown.

EGCG is the predominant flavonoid in tea and constitutes 10–15% of green tea leaves by dry weight. We, therefore, tested the effect of a freshly prepared green tea infusion on the infectivity of adenovirus. This gave an IC_{50} at 1/8 dilution of the infusion, which corresponds to 225 μM EGCG in the infusion. Storing the infusion at 4 °C or room temperature for 7 days had little effect on its efficacy.

How does EGCG and green tea inactivate adenovirions? Particle integrity was tested by density gradient centrifugation in CsCl after exposure (15 min, followed by dialysis) to different concentrations of EGCG and green tea infusion. Up to a concentration of 200 μM EGCG, neither agent affected the normal banding at 1.34 g/ml of the virions. Beyond that, the band diminished in a dose–response, and disappeared completely at 500 μM. Viral DNA became sensitive to digestion by micrococcal nuclease in the same dose-range. Adenain activity in the treated virions was also verified as described before (Tremblay et al., 1983). Compared to mock-treated virions, adenain activity was reduced to 40% by 250 μM EGCG and to 15% by green tea infusion containing the same concentration of EGCG.

The plasma concentration of EGCG after 2–3 cups of tea has been reported to be 0.1–0.3 μM in man (Cao and Cao, 1999; Lee et al., 2002). Another report determined the plasma concentration two hours after the consumption of 1.5 gm of tea in 500 ml of water at 0.218 μM (Yang et al., 1998a). The half-life was a surprising 5.0–5.5 h. These values are too remote compared to the IC_{50} of 225 μM antiviral effect obtained in vitro, to afford protection against the virus to green tea drinkers.

3.3. Inhibition of adenovirus endopeptidase

The effect of green tea catechins on the activity of adenain was tested in an in vitro assay. The fluorescent peptide substrate assay which we have used successfully in the past

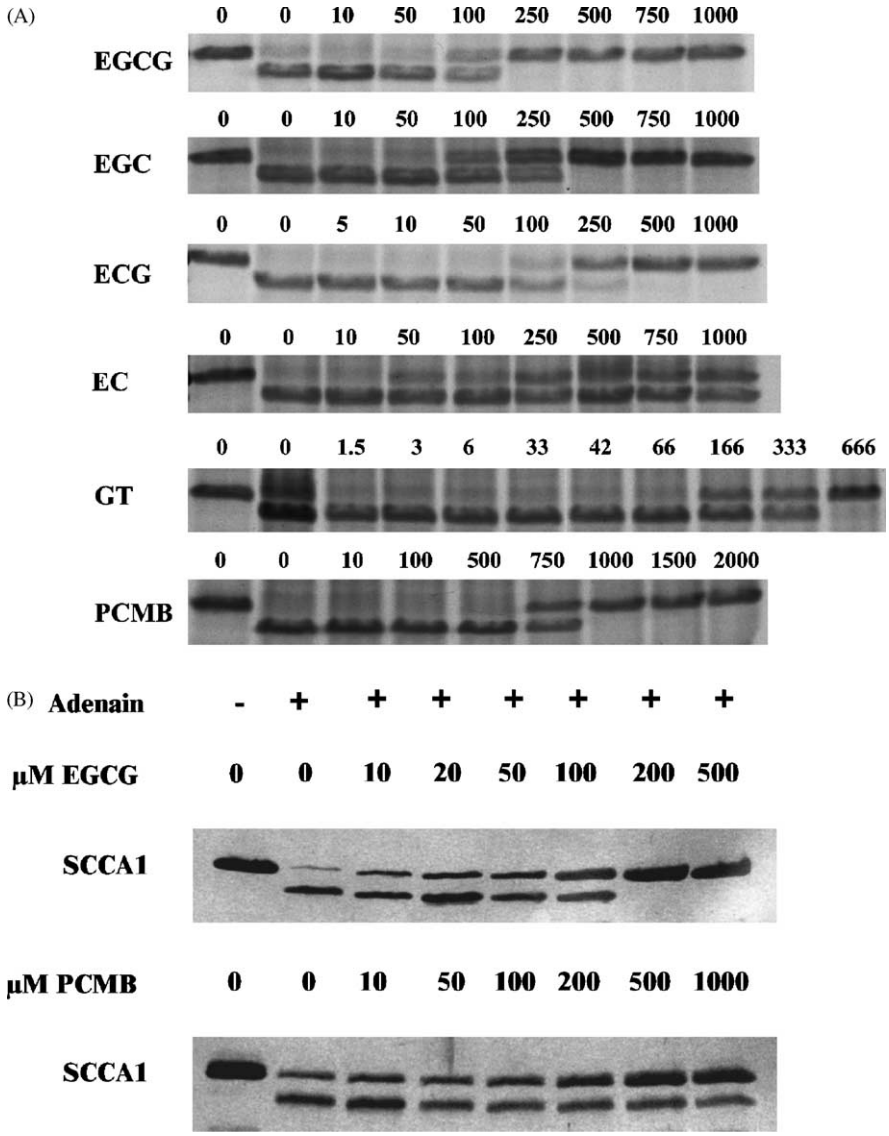

Fig. 4. The effect of tea catechins on adenain activity. Panel A: the reaction mixtures contained labeled ts1 proteins (including 10 μg pre-VII protein), 0.5 μg of recombinant adenain (all lanes except first), 40 μM pVIc activating peptide, and different concentrations of inhibitors (in μM above lanes). GT-100, green tea ("gun powder" infusion in 100 ml water). Reactions were incubated at 37 °C for 18 h, stopped by boiling in lysing solution, subjected to SDS–PAGE, and autoradiographed. Only the pre-VII and cleaved VII region of the gel is shown. Panel B: the effect of EGCG and PCMB on adenain activity. The reaction mixtures contained 1 μg of SCCA1, 0.5 μg of recombinant adenain (all lanes except first), 40 μM pVIc activating peptide, and different concentrations of EGCG (in μM above lanes). Reactions were incubated at 37 °C for 18 h, stopped by boiling in lysing solution, subjected to SDS–PAGE and stained with silver nitrate.

to study adenain could not be used due to the quenching of fluorescence by the catechins (Ruzindana-Umunyana and Weber, 2001; Diouri et al., 1995). We have therefore used our second assay based on the cleavage of the viral precursor protein PVII (Keyvani-Amineh et al., 1995). The effect of the catechins varied widely (Fig. 5, panel A). Quantitation was by densitometric scanning of the lanes, taking the second lane as control. 50% inhibition of adenain activity required 109 μM EGCG, followed by 143 μM ECG, 152 μM EGC and 1000 μM EC. A green tea infusion, which contains all four catechins but principally EGCG, yielded an IC_{50} of 200 μM, in terms of its EGCG concentration (Table 2). With the exception of EC, the catechins appear to be more efficient inhibitors of adenain activity than the standard cysteine protease inhibitor PCMB, which gave an IC_{50} of 714, but less efficient than against matrix metalloproteases (Table 2). The seven-fold greater efficacy of EGCG over PCMB was surprising. To confirm this difference and to rule out a bias by adenain due to the adenoviral protein substrate, we performed another assay using a foreign protein substrate. With the squamous cell carcinoma antigen (SCCA1) as substrate the IC_{50} for EGCG and PCMB were 75 and 500 μM, respectively (Fig. 5, panel B). These values and the ratio were similar to those above, thus confirming the greater

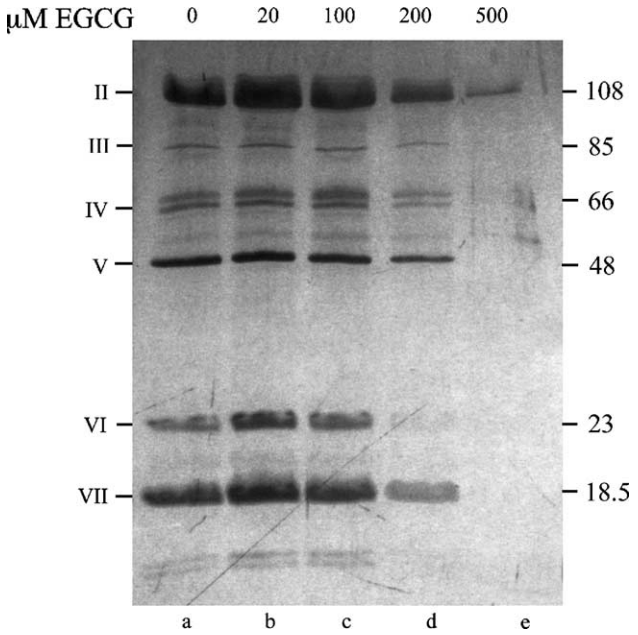

Fig. 5. Effect of EGCG on the production and polypeptide composition of virus particles. EGCG was added to the medium of infected cells at 18 h post-infection and at 48 h virus was purified from the combined medium plus cells by two cycles of density gradient centrifugation in CsCl. The material at 1.34 gm/ml density was collected, dialyzed and subjected to SDS–PAGE and stained with coomassie blue. The viral proteins are identified at the left, and the corresponding molecular weights at the right of the figure.

efficacy of EGCG as an inhibitor of adenain, in comparison to PCMB.

3.4. Conclusion

In the absence of effective anti-adenoviral chemotherapies we studied the antiviral effects of natural compounds, the catechins in Chinese green tea. These compounds, and particularly the principal component of green tea, epigallocatechin-3-gallate (EGCG) were chosen, based on the reported anti-protease and anti-HIV and anti-influenza virus effects (Garbisa et al., 1999, 2001; Demeule et al., 2000; De Clercq, 2000; Yamaguchi et al., 2002; Nakayama et al., 1993). EGCG was effective in inhibiting virus growth in cell culture with a therapeutic index of 22. EGCG and green tea itself have antiviral properties manifested at several levels including direct inactivation of virus particles, inhibition of intracellular virus growth, and inhibition of the viral protease, adenain, in vitro. However, the anti-adenoviral effects measured in vitro and ex vivo were at drug concentrations well above the plasma concentrations of EGCG in green tea drinkers to presume to afford protection against adenovirus infection. All of the present experiments were conducted with human adenovirus type 2, the most commonly used serotype. Whether EGCG is also effective against other adenovirus serotypes remains to be determined. Because the present experiments suggest that the viral protease, or adenain, is the target of EGCG and because the amino acid sequence is highly conserved among adenoviruses, we expect all adenoviruses to be sensitive to EGCG.

Table 2
Comparison of the IC_{50} values for the inhibition of matrix metalloproteases[a] and adenain[b]

Agent	IC_{50} (μM)		
	MMP-2	MMP-9	Adenain
Epigallocatechin gallate (EGCG)	6	0.3	109
Epigallocatechin (EGC)	95	28	152
Epicatechin gallate (ECG)	–	–	143
Epicatechin (EC)	–	–	1000
Green tea infusion (GT-100)[c]	–	–	200
p-Chloromercuribenzoate (PCMB)	–	–	714

[a] From Demeule et al. (2000).
[b] Calculated from data in Fig. 5.
[c] One gram of "Gunpowder tea" in 100 ml boiling nanopure water for 10 min.

Acknowledgements

This research was supported by grant MT4164 to J.M.W. from the Canadian Institutes of Health Research.

References

Balakirev, M.Y., Jaquinod, M., Haas, A.L., Chroboczek, J., 2002. Deubiquitinating function of adenovirus proteinase. J. Virol. 76 (12), 6323–6331.
Cai, Y., Anavy, N.D., Chow, H.-H.S., 2002. Contribution of presystemic hepatic extraction to the low oral bioavailability of green tea catechins in rats. Drug Metab. Disposition 30, 1246–1249.
Cao, Y., Cao, R., 1999. Angiogenesis inhibited by drinking tea. Nature 398, 381.
Chen, Z.P., Schell, J.B., Ho, C.-T., Chen, K.Y., 1998. Green tea epigallocatechin gallate shows a pronounced growth inhibitory effect on cancerous cells but not on their normal counterparts. Cancer Lett. 129, 173–179.
Cornish, J.A., Murray, H., Kemp, G.D., Gani, D., 1995. Specific inhibitors of the adenovirus type 2 protease based on substrate-like tetrapeptide nitrites. Bioorg. Med. Chem. Lett. 5, 25–30.
De Clercq, E., 1993. Therapeutic potential of HPMPC as an antiviral drug. Rev. Med. Virol. 8, 261–272.
De Clercq, E., 2000. Current lead natural products for the chemotherapy of human immunodeficiency virus (HIV) infection. Med. Res. Rev. 20, 323–349.
Demeule, M., Brossard, M., Page, M., Gingras, D., Beliveau, R., 2000. Matrix metalloprotease inhibition by green tea catechins. Biochim. Biophys. Acta 36094, 1–10.
Ding, J., McGrath, W.J., Sweet, R.M., Mangel, W.F., 1996. Crystal structure of the human adenovirus protease with its 11 amino acid cofactor. EMBO J. 15, 1778–1783.
Diouri, M., Geoghegan, K.F., Weber, J.M., 1995. Functional characterization of the adenovirus protease using fluorogenic substrates. Protein Peptide Lett. 6, 363–370.
Doronin, K., Kuppuswamy, M., Toth, K., Tollefson, A.E., Krajesi, P., Krougliak, V., Wold, W.S.M., 2001. Tissue-specific, tumor-selective,

replication-competent adenovirus vector for cancer gene therapy. J. Virol. 75 (7), 3314–3324.
Garbisa, S., Biggin, S., Cavallarin, N., Sartor, L., Benelli, R., Albini, A., 1999. Tumor invasion: molecular shears blunted by green tea. Nat. Med. 5 (11), 1216.
Garbisa, S., Sartor, L., Biggin, S., Salvato, B., Benelli, R., Albini, A., 2001. Tumor gelatinases and invasion inhibited by the green tea flavanol epigallocatechin-3-gallate. Cancer 91 (4), 822–832.
Guo, N., Ruiz, A., Rando, R.R., Bok, D., Gudas, L.J., 2000. Esterification of all-trans-retinol in normal human epithelial cell strains and carcinoma lines from oral cavity, skin and breast: reduced expression of lecithin:retinol acyltransferase in carcinoma lines. Carcinogenesis 21 (11), 1925–1933.
Haneda, E., Furuya, T., Asai, S., Morikawa, Y., Ohtsuki, K., 2000. Biochemical characterization of casein kinase II as a protein kinase responsible for stimulation of HIV-1 protease in vitro. Biochem. Biophys. Res. Commun. 275 (2), 434–439.
Horwitz, M. 1996. Adenoviruses. In: Fields, B.N. et al. (Eds.), Virology, vol.2. Lippincott-Raven, Philadelphia.
Jankun, J., Selman, S.H., Swiercz, R., 1997. Why drinking green tea could prevent cancer? Nature 387, 561.
Keyvani-Amineh, H., Labrecque, P., Cai, F., Carstens, E.B., Weber, J.M., 1995. Adenovirus protease expressed in insect cells cleaves adenovirus proteins, ovalbumin and baculovirus protease in the absence of activating peptide. Virus Res. 37, 87–97.
Lee, M.-J., Maliakal, P., Chen, L., Meng, X., Bondoc, F.Y., Prabhu, S., Lambert, G., Mohr, S., Yang, C.S., 2002. Cancer Epidem. Biomarkers Prev. 11, 1025–1032.
Li, S., Hochstrasser, M., 1999. A new protease required for cell-cycle progression in yeast. Nature 398, 246–251.
Lopez-Otin, C., Simon-Mateo, C., Martinez, L., Vinuela, E., 1989. Gly-Gly-X, a novel consensus sequence for the proteolytic processing of viral and cellular proteins. J. Biol. Chem. 264, 9107–9110.
Mangel, W.F., McGrath, W.J., Brown, M.T., Baniecki, M.L., Barnard, D.L., Pang, Y.-P., 2001. A new form of antiviral combination therapy predicted to prevent resistance from arising and a model system to test it. Curr. Med. Chem. 8, 933–939.
Mentel, R., Kinder, M., Wegner, U., von Janta-Lipinski, M., Matthes, E., 1997. Inhibitory activity of 3′-fluoro-2′-deoxythymidine and related nucleoside analogues against adenoviruses in vitro. Antiviral Res. 34 (3), 113–119.
Mentel, R., Kurek, S., Wegner, U., Janta-Lipinski, M., Gurtler, L., Matthes, E., 2000. Inhibition of adenovirus DNA polymerase by modified nucleoside triphosphate analogs correlate with their antiviral effects on cellular level. Med. Microbiol. Immunol. 189 (2), 91–95.
Mentel, R., Wegner, U., 2000. Evaluation of the efficacy of 2′,3′-dideoxycytidine against adenovirus infection in a mouse pneumonia model. Antiviral Res. 47 (2), 79–87.
Morre, D.J., Bridge, A., Wu, L.-Y., Morre, D.M., 2000. Preferential inhibition by (-)-epigallocatechin-3-gallate of the cell surface NADH oxidase and growth of transformed cells in culture. Biochem. Pharmacol. 60, 937–946.
Mukhtar, H., Ahmad, N., 1999. Green tea in chemoprevention of cancer. Toxicol. Sci. 52 (2 Suppl), 111–117.
Nakayama, M., Suzuki, K., Toda, M., Okubo, S., Hara, Y., Shimamura, T., 1993. Inhibition of the infectivity of influenza virus by tea polyphenols. Antiviral Res. 21 (4), 289–299.
Nevels, M., Tauber, B., Spruss, T., Wolf, H., Dobner, T., 2001. Hit-and-run transformation by adenovirus oncogenes. J. Virol. 75, 3089–3094.
Pang, Y.-P., Xu, K., Kollmeyer, T.M., Perola, E., McGrath, W.J., Green, D.T., Mangel, W.F., 2001. Discovery of a new inhibitor lead of adenovirus protease: steps toward selective, irreversible inhibitors of cysteine proteases. FEBS Lett. 502, 93–97.
Precious, B., Russell, W.C., 1985. Growth, purification and titration of adenoviruses. In: Mahy, B.W.J. (Ed.), Virology: A Practical Approach. IRL, Oxford, England, pp. 193–205.
Qualikene, W., Lamoureux, L., Weber, J.M., Massie, B., 2000. Protease-deleted adenovirus vectors and complementing cell-lines: applications of single-round replication mutants for vaccination and gene therapy. Hum. Gene Ther. 11, 1341–1353.
Ruzindana-Umunyana, A., Sircar, S., Weber, J.M., 2000. The effect of mutant peptide cofactors on adenovirus protease activity and virus infection. Virology 270, 173–179.
Ruzindana-Umunyana, A., Weber, J.M., 2001. Interactions of human lacrimal and salivary cystatins with adenovirus endopeptidase. Antiviral Res. 51, 203–214.
Sachinidis, A., Seul, C., Seewald, S., Ahn, H.-Y., Ko, Y., Vetter, H., 2000. Green tea compounds inhibit tyrosine phosphorylation of PDGF β-receptor and transformation of A172 human glioblastoma. FEBS Lett. 471, 51–55.
Sircar, S., Keyvani-Amineh, H., Weber, J.M., 1996. Inhibition of adenovirus infection with protease inhibitors. Antiviral Res. 30, 147–153.
Sircar, S., Ruzindana-Umunyana, A., Neugebauer, W., Weber, J.M., 1998. Adenovirus endopeptidase and papain are inhibited by same agents. Antiviral Res. 40, 45–51.
Weber, J.M. 1999. Role of endopeptidase in adenovirus infection. In: P. Seth (Ed.), Adenoviruses: From Basic Research to Gene Therapy Application. R.G. Landes Company.
Whickam, T.J., 2000. Targeting adenovirus. Gene Ther. 7, 110–114.
Williams, S.N., Shih, J., Guenette, D.K., Brackney, W., Denison, M.S., Pickwell, G.V., Quattrochi, L.C., 2000. Comparative studies on the effects of green tea extracts and individual tea catechins on human CYP1A gene expression. Chemico-Biol. Interact. 128, 211–229.
Yamaguchi, K., Honda, M., Ikigai, H., Hara, Y., Shimamura, T., 2002. Inhibitory effects of (-)-epigallocatechin gallate on the life cycle of human immunodeficiency virus type 1 (HIV-1). Antiviral Res. 53, 19–34.
Yang, C.S., Chen, L., Lee, M.J., Balentine, D., Kuo, M.C., Schantz, S.P., 1998a. Blood and urine levels of tea catechins after ingestion of different amounts of green tea by human volunteers. Cancer Epidem. Biomarkers Prev. 7, 351–354.
Yang, G.Y., Liao, J., Kim, K., Yurkow, E.J., Yang, C.S., 1998b. Inhibition of growth and induction of apoptosis in human cancer cell lines by tea polyphenols. Carcinogenesis 19, 611–616.
Yang, C.S., Wang, Z.Y., 1993. Tea and cancer. J. Natl. Cancer Inst. 85, 1038–1049.