Eur J Nutr (2010) 49:227–234
DOI 10.1007/s00394-009-0068-0

ORIGINAL CONTRIBUTION

Prevention of oxidative DNA damage in inner organs and lymphocytes of rats by green tea extract

Nina Kager · Franziska Ferk · Michael Kundi · Karl-Heinz Wagner · Miroslav Mišík · Siegfried Knasmüller

Received: 4 June 2009 / Accepted: 7 October 2009 / Published online: 23 October 2009

Abstract
Background Consumption of green tea (GT) is associated with decreased incidences of specific forms of cancer in humans and it was postulated that its antioxidant (AO) properties may account for these effects. The evidence for AO effects of GT is mainly based on the results from in vitro experiments and on animal studies in which protection against chemically induced damage was monitored.
Aim of the study The goal of the study was the investigation of the prevention of strand breaks and DNA migration attributable to endogenous oxidation of bases by GT extract (GTE) in inner organs and lymphocytes of untreated rats. In addition, immunological parameters and biochemical markers were monitored.
Methods DNA migration was measured in hepatocytes, colonocytes and lymphocytes after consumption of a low (1.3 mg/kg bw per day, 5 days) and a high dose (6.5 mg/kg bw per day, 5 days) of GTE in COMET assays ($n = 5$ animals per group). In addition, immunological parameters (TNF-α, IFN-γ, IL-4 and IL-10), the total AO capacity and oxidized low-density lipoproteins were determined in plasma.
Results No evidence for reduction in DNA damage was found with a lower dose, whereas with the higher dose, reduction in DNA migration attributable to formamidopyrimidine-DNA-glycosylase sensitive lesions (oxidized purines) and endonuclease III-sensitive sites (oxidized pyrimidines) (58 and 73%) was observed in lymphocytes; also, in colonocytes (reduction in FPG-sensitive sites by 46%) and hepatocytes (decrease in Endo III-sensitive sites by 74%) protective effects were found, while none of the other parameters was altered.
Conclusions Our results show that a dose of GTE, which is equivalent to consumption of 500 ml GT/p/day in humans protects lymphocytes and to a lesser extent inner organs against oxidative DNA damage, while no effect was seen with a lower dose corresponding to an uptake of 100 ml/p/day.

Keywords Green tea · Single-cell gel electrophoresis assay · Oxidative DNA damage · Biochemical parameters · Immunological parameters

Abbreviations

AO	Antioxidative
Endo III	Endonuclease III
FPG	Formamidopyrimidine-DNA-glycosylase
GT	Green tea
GTE	Green tea extract
IFN-γ	Interferon gamma
IL	Interleukin
OxoLDL	Oxidized low-density lipoprotein
SCGE	Single-cell gel electrophoresis

N. Kager · F. Ferk · M. Mišík · S. Knasmüller (✉)
Department of Medicine I, Institute of Cancer Research,
Medical University of Vienna,
Borschkegasse 8a, 1090 Vienna, Austria
e-mail: siegfried.knasmueller@meduniwien.ac.at

M. Kundi
Center for Public Health, Institute of Environmental Health,
Medical University of Vienna,
Kinderspitalgasse 15, 1090 Vienna, Austria

K.-H. Wagner
Department of Nutritional Sciences, University of Vienna,
Althanstraße 14, 1090 Vienna, Austria

TEAC Trolox equivalent antioxidative capacity
TNF-α Tumor necrosis factor alpha

Introduction

Numerous epidemiological studies indicate that the consumption of green tea (GT) is inversely related to the incidence of breast, prostate and gastrointestinal cancer, coronary heart disease as well as neurogenerative disorders (for review see Cabrera et al. [1]). In all these diseases, reactive oxygen species are involved and it was postulated that the antioxidant (AO) properties of GT may account for its beneficial health effects [2].

The results of in vitro experiments show that the AO capacity of GT is higher than that of common fruits and vegetables [3] and correlates with its catechin content [4]. Also, the results of a few animal and human studies showed that GT protects against oxidative DNA damage of macromolecules and increases the AO status (for reviews see [5, 6]). It was shown in experiments with laboratory rodents that tea prevents chemically induced formation of oxidised guanosine (for review see [5]), but it has not been investigated so far if it prevents also the formation of single and double-strand breaks and DNA migration due to oxidation of DNA bases in inner organs.

Therefore, we performed in vivo experiments in which we studied the effect of green tea extract (GTE) on DNA migration in colons, livers and peripheral blood cells in single-cell gel electrophoresis (SCGE) experiments with rats. We included also measurements with colon cells, as a number of studies indicate that the intake of tea is inversely related to the incidence of gastrointestinal cancer in humans [7, 8]. In contrast to the earlier studies, we used chemically untreated animals that reflect the situation in healthy humans.

SCGE experiments are based on the determination of DNA migration in an electric field and enable to monitor damage in inner organs [9]. Furthermore, we also included measurements of immunological parameters (tumor necrosis factor alpha, interferon γ and interleukins 4 and 10) which are considered to play an important role in the human health status, as it is known that AOs may affect immunological functions. In addition, we determined in plasma the total AO capacity (TEAC), a general marker of the oxidant status in humans [10] and oxidized low-density lipoprotein (oxoLDL) levels which reflect the improvement of the AO status in humans by dietary factors and are assumed to be related to atherosclerosis [11].

Materials and methods

Chemicals

Low and normal melting agarose were obtained from Invitrogen Life Technologies (Paisley, Scotland). Trizma base, 2,2′-azino-bis(3-ethylbenzthiazoline)-6-sulfonic acid (ABTS), bovine serum albumin fraction V, ethidium bromide, Histopaque-1077, myoglobin, RPMI 1640, Sephadex G-15, Triton X-100, trypan blue, organic and inorganic salts were purchased from Sigma-Aldrich (Steinheim, Germany). Dimethylsulfoxide was purchased from Merck (Darmstadt, Germany). 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) was purchased from Fluka (Buchs, Switzerland), Na–heparin was from Ebewe Pharma (Unterach, Austria).

Formamidopyrimidine-DNA-glycosylase (FPG) and endonuclease III (Endo III) were obtained from M. Dušínska (Slovak Medical University, Bratislava, Slovakia).

Lyophilized GTE (Sunphenon 90M) was obtained from Taiyo International Inc. (Minneapolis, USA). According to the manufacturers, it contains approximately 80% polyphenols and not less than 75% of total catechins, its epigallocatechin-3-gallate (EGCG) content is approximately 45%.

The enzyme-linked immunosorbent assay test kits (ELISA) for the measurement of the immunological parameters were obtained from BD OptEIA, BD Biosciences Pharmingen (San Diego, Canada) and the Lipid-Ox fluorescence test came from Dr. Franz Tatzber KEG (Klosterneuburg, Austria).

Animals

All experiments were carried out with male 5–6-weeks-old Him-OFA rats (body weight 250 ± 10 g) which were obtained from the breeding facility of the Medical University Vienna (Himberg, Austria). Before the start of the experiments, the animals were allowed to acclimatize for 1 week and were housed in plastic cages (Macrolon type III, Techniplast GmbH, Hohenpeißenberg, Germany) under standard conditions (24 ± 1°C, humidity 50 ± 5%, 12 h light/dark cycle) and were fed with a standard diet (R/M-H, Ssniff, Soest, Germany).

Treatment of the animals

The study was approved by the Ethics Committee of the Medical University of Vienna. Each treatment group consisted of five animals. The GTE was dissolved in tap water, the solutions were made fresh every day. GT solution or the solvent (water) were given to the animals orally (by gavage; 1.3 ml per animal), for more than 5 days. The low dose group received 1.3 mg/kg bw of the lyophilized

powder per day, the high dose group received 6.5 mg/kg bw per day. The GTE amounts which we used correspond to consumption of 100 and 500 ml GT in humans. The calculations are based on the EGCG content of the extract which contained 45 mg EGCG per 100 mg. The average amount of this catechin in fresh GT brews made from 1.5 g of dry leaves per 100 ml is 40–50 mg per 100 ml [12–14].

The animals were weighed before and after the treatment with GTE. The weight gain in the three groups were 7.8 ± 1.7% in controls, 6.9 ± 1.4% in the low dose group and 8.9 ± 0.4% in the high dose group; the differences between the groups are statistically not significant.

SCGE experiments

24 h after the last treatment, the animals were killed by decapitation. Subsequently, the livers and colons were removed and transferred to PBS (pH 7.4). Blood was collected in lithium-heparin tubes (Becton Dickinson, Plymouth, UK).

The nuclei were isolated from the livers by homogenization of the hepatic tissue with a Potter–Elvehjem type homogenizer (Braun, Melsungen, Germany) and subsequent centrifugation, colon cells were collected by scraping (for details see Sasaki et al. [9]). Peripheral lymphocytes were isolated from the blood by density gradient centrifugation with Histopaque-1077 in Accuspin™ tubes (Sigma-Aldrich, Steinheim, Germany) according to the manufacturers.

All subsequent experiments were carried out as described in the protocol of Collins et al. [15] according to the international guidelines [16]. The cells were transferred to agarose-coated slides (1.5% normal melting and 0.5% low melting agarose) and lysed in buffer (pH 10) for 1 h. After 20 min unwinding and 20 min electrophoresis (300 mA, 25 V at 4°C, pH > 13), the gels were stained with ethidium bromide (20 µg/ml).

In addition, nuclei were treated after lysis with the lesion-specific enzymes FPG and Endo III. To establish the optimal amounts of the enzymes, we conducted calibration experiments with different dilutions of the enzymes prior to the main study (data not shown). We found optimal activity with Endo III with a 1:9,000 dilution of the stock solution (1.0 µg enzyme per ml), and with a 1:1,000 FPG dilution. After lysis, the slides were washed twice in enzyme reaction buffer (pH 8.0) for 8 min. Subsequently, 50 µl of FPG or Endo III solution were added to the nuclei. For FPG, the incubation time was 30 min, for Endo III 45 min (37°C). After 40 min unwinding and 30 min electrophoresis (300 mA, 25 V at 4°C, pH > 13), the gels were stained with ethidium bromide (20 µg/ml). For each endpoint, three slides were made in parallel and 150 cells were analyzed for comet formation (endpoint: percentage DNA in tail) by the use of an automated image analysis system (Comet Assay IV, Perceptive Instruments, UK). The extent of DNA migration attributable to the formation of oxidized bases was calculated by subtracting values obtained with the buffers from values obtained with the individual enzymes.

Measurement of the immunological parameters

The different parameters (TNF-α, IFN-γ, IL-4, IL-10) were determined in plasma by the use of commercially available ELISA kits after the instructions of the manufacturer. The measurements were carried out with an ELISA reader (FLUOstar Optima, BMG Labtechnologies, Offenburg, Germany). All measurements were carried out in duplicate.

Determination of the total AO capacity

Trolox equivalent antioxidative capacity was analyzed in plasma photometrically ($\lambda = 734$ nm) after iron-induced oxidation after the protocol of Miller et al. [17]. The procedure is based on the ability of an antioxidant to scavenge blue-green colored 2,2′-azinobis(3-ethtylbenzthiazoline-6-sulfonic acid) (ABTS) radicals, which are generated by the peroxidase activity of metmyoglobin in the presence of the sample. 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carbonic acid (Trolox, a vitamin E analog) solution was used to establish the standard curves.

Measurement of low-density lipoprotein oxidation

Oxidized low-density lipoprotein was measured fluorimetrically ($\lambda = 450$ nm) [18] with a commercially available kit after the instructions of the manufacturer with FLUOstar Optima reader (BMG Labtechnologies, Offenburg, Germany). In this test, the biological lipid system is preloaded with a fluorescent analog of phosphatidylcholine. When externally added, the respective phospholipid label localizes to the surface monolayer of a lipoprotein. When the fluorophore is oxidized, it decomposes which leads to a continuous decrease in the fluorescence intensity [18]. As described in the study of Mayer et al. [19], the fluorophore binds preferentially to the LDL fraction in plasma and can be, therefore, used to assess its concentration.

Statistical analysis

For the statistical evaluation of the SCGE experiments, three medians were calculated for each animal, and from these, the mean values were computed for each endpoint. To obtain the homogeneity of variance, the percentage DNA in tail (% tail intensity) was arcsine transformed.

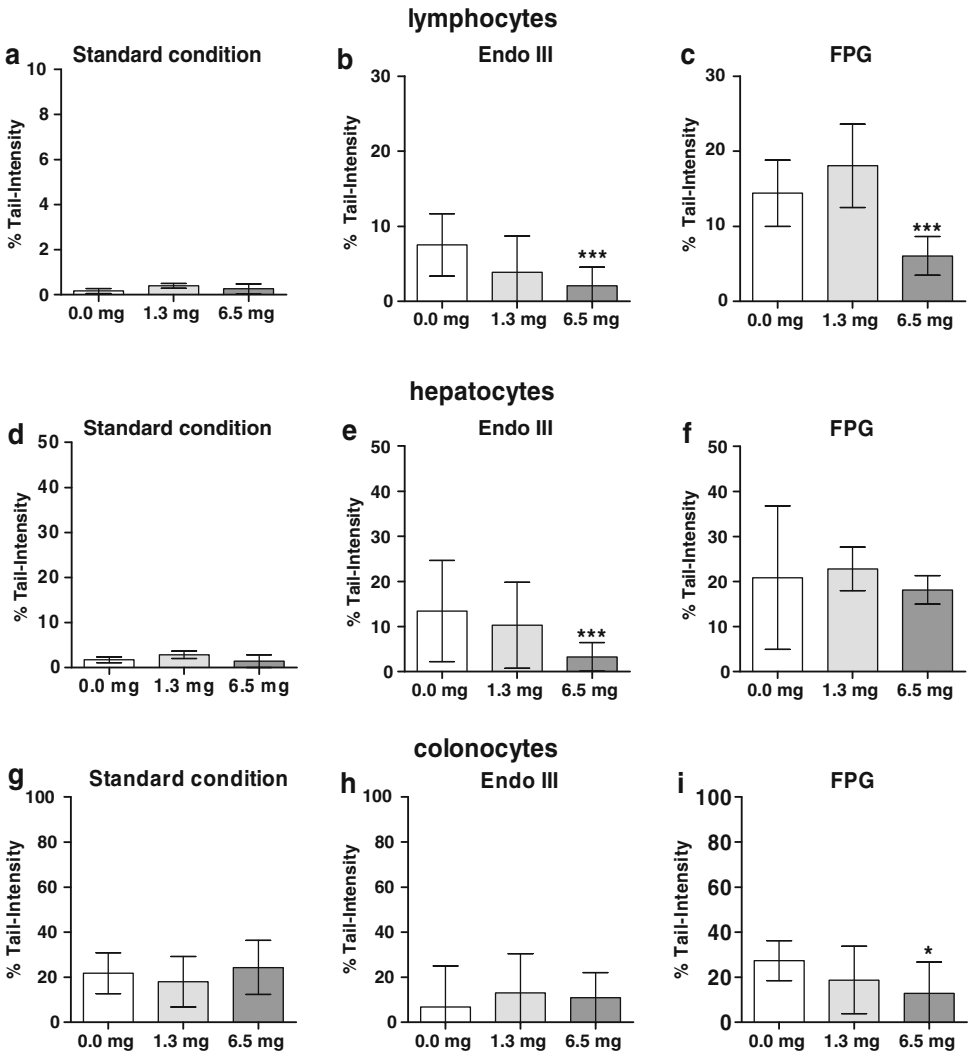

Fig. 1 **a–i** Impact of oral administration of GTE to rats on DNA migration in peripheral lymphocytes, hepatocytes and colonocytes. The animals received GTE for a period of 5 days by gavage. Subsequently, the animals were killed by decapitation, blood was collected, the organs removed and nuclei isolated as described in "Materials and methods". DNA migration was determined under standard conditions and after treatment of the nuclei with lesion-specific enzymes (FPG, Endo III). From each organ and from the lymphocytes, three slides were made in parallel for each endpoint and 50 nuclei were randomly analyzed per slide. *Bars* represent mean ± standard deviations of results obtained with five animals per group and show the net enzyme sensitive sites (values obtained with the enzyme buffer were subtracted from the values obtained with the respective enzyme). *Stars* indicate statistical significance: $*P < 0.05$, $***P < 0.001$

Outliers were removed with the Dixon's test. The analysis of variance was conducted by application of a mixed model with animals as random factor and condition as between animal factors. Comparisons between groups of primary interest were done by linear contrasts and contrasts were considered significant if $P < 0.05$. The homogeneity of variances was tested by Levene's test. Residuals were assessed for normality by Lilliefors test. For these tests, the significance level was set at 0.01.

Statistical analyses of the immunological and biochemical parameters (TEAC and oxoLDL) were carried out by one-way analysis of variances. Differences were considered significant if $P < 0.05$.

Results

The results of the SCGE experiments with GTE are shown in Fig. 1. It can be seen that the extent of damage was in the control group in the colons in general higher than in the blood cells and in the hepatic tissue. This observation is in agreement with findings from earlier studies [9, 20]. The treatment of the nuclei with the lesion-specific enzymes led to a pronounced increase in comet formation indicating that the enzymes were active.

With the lower GTE dose, no indication of a protective effect was seen in any of the organs and also not in the blood cells. In contrast, clear effects were seen with the higher dose. The most pronounced reduction in DNA damage was observed in the lymphocytes in which comet formation due to FPG-sensitive lesions was decreased by 58% ($P < 0.001$) and Endo III lesions were reduced by 73% ($P < 0.001$); also in the inner organs, significant protective effects were observed, i.e. a 74% decrease in Endo III-sensitive lesions ($P < 0.001$) was detected in the hepatic tissue and in the colon cells a decrease by 46% of FPG-sensitive sites ($P < 0.05$) was found.

Table 1 Impact of the treatment of rats with green tea extract on immunological and biochemical parameters

Endpoint (unit)	Controls Mean ± SD	GTE (1.3 mg) Mean ± SD	GTE (6.5 mg) Mean ± SD
Immunological parameters			
TNF-α (pg/ml)	109.1 ± 35.2	102.3 ± 35.3	120.4 ± 32.5
IFN-γ (pg/ml)	139.6 ± 35.1	141.4 ± 48.1	144.3 ± 42.1
IL-4 (pg/ml)	55.3 ± 9.2	69.6 ± 14.2	68.7 ± 11.3
IL-10 (pg/ml)	105.5 ± 15.4	137.5 ± 12.3	151.5 ± 27.3
Biochemical parameters			
TEAC (mM Trolox equivalent)	0.64 ± 0.11	0.55 ± 0.07	0.63 ± 0.09
oxoLDL (lagPhase min)	14.67 ± 0.58	15.6 ± 1.52	15.6 ± 2.07

The experiment was carried out as described in the legend of Fig. 1 and in "Materials and methods"

All measurements were conducted with plasma

Values indicate mean ± standard deviations obtained with five animals per group

Table 1 summarizes the results of the biochemical measurements. It can be seen that none of the different parameters investigated was significantly altered by GTE treatment.

Discussion

Taken together, the results of the present study show for the first time that GTE protects healthy animals against oxidative DNA damage when a sufficiently high dose is administered.

As described above, the effects were more pronounced in blood and liver cells than in the colonocytes. This finding was unexpected, as the concentrations of AO components of GT in the gastrointestinal tract are higher than in the inner organs. However, a number of investigations indicate that GT induces transcription factors (for review, see Na und Surh [21]) and it is conceivable that the extent of this cell signaling-mediated responses is organ specific [22].

A recent comparative evaluation of the results of human intervention trials in which different endpoints were monitored showed that the overlap of protection against the prevention of oxidized purines and pyrimidines is very poor. Protection against Endo III-sensitive sites was seen in a higher number of trials [23]. Therefore, it is not likely that the lack of protective effects in both endpoints (prevention of oxidation of purines and pyrimidines) in inner organs is due to the study design, for example, due to the number of animals which was higher than that recommended in the guidelines of Tice et al. [24].

As mentioned above, a number of animal studies have been published which concern the prevention of chemically induced formation of 8-hydroxy-2-deoxy-guanosine (8-OHdG) by GT and its constituents. In this context, it is notable that it has been shown by Gedik et al. [25] in a small human study that the formation of this purine base corresponds to DNA migration caused by FPG-sensitive sites. Clear protective effects (i.e. reduction 8-OHdG formation) were seen, for example, in experiments with mice in lungs [26] and with rats in livers [27] and also in pancreatic tissue [28] of hamsters after the induction of oxidative stress with nitrosamines. In addition, protective effects were found in combination with the colon carcinogen dimethylhydrazine in colon cells [29], while no evidence for reduction in DNA migration was detected with the tea constituents, theorubigin and theafulvin [30]. Only one SCGE study with a GT component has been carried out according to our knowledge by Giovanelli et al. [31] in which the effect of theorubigin was investigated in rats fed a high fat diet, and no reduction in DNA migration due to Endo III-sensitive sites in colon mucosa cells was detected.

The results from a human study showed that the frequencies of micronuclei in peripheral lymphocytes are reduced in smokers after consumption of more than 2 g GT per day [32]. Also in another study in which sister chromatid exchange rates were monitored in smokers and non-smokers who consumed 2–3 cups over 6 months, a significant protective effect was detected in the same indicator cells [33], while no evidence for protection was found in non-smokers in a recent intervention study with GT-supplemented bread in which DNA damage was monitored in blood cells of healthy individuals [34].

It is interesting that the extent of DNA damage seen in the lymphocytes of rats was much lower as that seen in human studies and also that the level of oxidative base damage was substantially higher. Also in a number of other studies with rodents, the baseline damage found with lymphocytes was quite low, although slightly higher as that we found in our study [35, 36]. The relatively high extent of oxidative damage may be due to the increased energy metabolism of rats compared with humans and/or other factors (e.g. differences of the size of the nuclei).

As mentioned above, no impact of GTE on the different immunological parameters was found in the present study (Table 1). One of the reasons for the lack of an effect of GTE on the IL and on TNF-α and IFN-γ levels may be that the study was conducted with healthy animals. In earlier investigations, significant reduction in the levels of these markers was seen in rodents after the induction of inflammations with lipopolysaccharide and bacterial toxins [14, 37], while no effects were seen in a colitis model with mice [38] and in a human trial with healthy individuals [39].

The impact of GT on oxoLDL levels has been investigated in a number of human studies and the results are highly controversial; in some, no alterations were found [40–42] while in others clear protective effects were detected [29, 33, 35]. Likewise, also investigations concerning the impact of GT on the TEAC value yielded inconsistent results [4, 42–44].

As described in the results section, we failed to detect alterations of the TEAC and of LDL oxidation by GTE while in the same experiments protection against oxidative DNA damage was observed. It is notable that a comprehensive analysis of studies in which different endpoints of the AO status were measured in parallel by Dotan et al. [45] showed that in general no correlations exist between makers of the AO capacity and oxidative damage of the genetic material. A similar conclusion was reached by Knasmüller et al. [22] and it was emphasized by the authors that the combined use of disease-related markers in studies with antioxidants will provide more relevant information.

Our findings of a DNA protective effect of GTE are not necessarily due to direct scavenging effects that were seen in a number of in vitro studies with GT and its constituents [1]. It is conceivable that prevention of DNA migration which we observed may be (at least partly) due to indirect effects such as reduced glucose uptake in the gastrointestinal tract leading to a hypocaloric status, that is associated with changes in the mitochondrial function and paralleled by decreased production of reactive oxygen species [46–48]. It is also notable that it has been shown that GT induces AO enzymes such as superoxide dismutase and glutathione-*S*-transferase and that these effects are triggered by induction of transcription factors [21]. Another mode of action is the modulation of repair processes, which was found with GT and its components in in vitro studies [49, 50]. The recent development of protocols which enable the determination of repair processes in SCGE assays [51] will allow to investigate this latter issue in future human experiments.

The consequences of a decreased basal level of oxidative damage of lymphocyte DNA in terms of health benefits are far from being clear. It has been shown in a number of investigations that occupational exposure associated with adverse health effects as well as infertility and different diseases (coronary heart diseases, diabetes, etc.) are characterized by increased levels of base oxidation in peripheral blood cells [52, 53]. In the case of cancer, recent findings showing that the intake of antioxidant supplementation does not lead to protection, but rather to increased risks made it questionable if protection of oxidative DNA damage is indeed paralleled by protection against malignant disease [52].

Our results indicate that the prevention of oxidative DNA damage takes place with a dose of GTE which is equivalent to daily consumption of 500 ml of GT by humans; this observation is relevant for the development of dietary recommendations and also for the production of GT-supplemented functional foods which are increasingly produced [54]. In this context, it is notable that results of several epidemiological studies indicate that cancer protective effects of GT are only seen after daily intake of five or more cups [55–57].

Acknowledgments We wish to thank Alfred Dutter for administration of the GTE to the animals by gavage and M. Dušínska (Institute of Preventive and Clinical Medicine, Bratislava, Slowakia) for providing the lesion-specific enzymes.

References

1. Cabrera C, Artacho R, Gimenez R (2006) Beneficial effects of green tea: a review. J Am Coll Nutr 25:79–99
2. Zaveri NT (2006) Green tea and its polyphenolic catechins: medicinal uses in cancer and noncancer applications. Life Sci 78:2073–2080
3. Cao G, Sofic E, Prior RL (1996) Antioxidant capacity of tea and common vegetables. J Agric Food Chem 44:3426–3431
4. Henning SM, Niu Y, Liu Y, Lee NH, Hara Y, Thames GD, Minutti RR, Carpenter CL, Wang H, Heber D (2005) Bioavailability and antioxidant effect of epigallocatechin gallate administered in purified form versus as green tea extract in healthy individuals. J Nutr Biochem 16:610–616
5. Frei B, Higdon JV (2003) Antioxidant activity of tea polyphenols in vivo: evidence from animal studies. J Nutr 133:3275S–3284S
6. Rietveld A, Wiseman S (2003) Antioxidant effects of tea: evidence from human clinical trials. J Nutr 133:3285S–3292S
7. Nakachi K, Matsuyama S, Miyake S, Suganuma M, Imai K (2000) Preventive effects of drinking green tea on cancer and cardiovascular disease: epidemiological evidence for multiple targeting prevention. Biofactors 13:49–54
8. Setiawan VW, Zhang ZF, Yu GP, Lu QY, Li YL, Lu ML, Wang MR, Guo CH, Yu SZ, Kurtz RC, Hsieh CC (2001) Protective effect of green tea on the risks of chronic gastritis and stomach cancer. Int J Cancer 92:600–604
9. Sasaki YF, Kawaguchi S, Kamaya A, Ohshita M, Kabasawa K, Iwama K, Taniguchi K, Tsuda S (2002) The comet assay with 8 mouse organs: results with 39 currently used food additives. Mutat Res 519:103–119
10. Somogyi A, Rosta K, Pusztai P, Tulassay Z, Nagy G (2007) Antioxidant measurements. Physiol Meas 28:R41–R55
11. Witztum JL, Steinberg D (1991) Role of oxidized low density lipoprotein in atherogenesis. J Clin Invest 88:1785–1792
12. Chan YC, Hosoda K, Tsai CJ, Yamamoto S, Wang MF (2006) Favorable effects of tea on reducing the cognitive deficits and brain morphological changes in senescence-accelerated mice. J Nutr Sci Vitaminol (Tokyo) 52:266–273
13. Hasegawa R, Chujo T, Sai-Kato K, Umemura T, Tanimura A, Kurokawa Y (1995) Preventive effects of green tea against liver oxidative DNA damage and hepatotoxicity in rats treated with 2-nitropropane. Food Chem Toxicol 33:961–970
14. Hisano M, Yamaguchi K, Inoue Y, Ikeda Y, Iijima M, Adachi M, Shimamura T (2003) Inhibitory effect of catechin against the

superantigen staphylococcal enterotoxin B (SEB). Arch Dermatol Res 295:183–189
15. Collins A, Dusinska M, Franklin M, Somorovska M, Petrovska H, Duthie S, Fillion L, Panayiotidis M, Raslova K, Vaughan N (1997) Comet assay in human biomonitoring studies: reliability, validation, and applications. Environ Mol Mutagen 30:139–146
16. Burlinson B, Tice RR, Speit G, Agurell E, Brendler-Schwaab SY, Collins AR, Escobar P, Honma M, Kumaravel TS, Nakajima M, Sasaki YF, Thybaud V, Uno Y, Vasquez M, Hartmann A (2007) Fourth International Workgroup on Genotoxicity Testing: results of the in vivo comet assay workgroup. Mutat Res 627:31–35
17. Miller NJ, Rice-Evans C, Davies MJ, Gopinathan V, Milner A (1993) A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in premature neonates. Clin Sci (Lond) 84:407–412
18. Hofer G, Lichtenberg D, Hermetter A (1995) A new fluorescence method for the continuous determination of surface lipid oxidation in lipoproteins and plasma. Free Radic Res 23:317–327
19. Mayer B, Schumacher M, Brandstatter H, Wagner FS, Hermetter A (2001) High-throughput fluorescence screening of antioxidative capacity in human serum. Anal Biochem 297:144–153
20. Majer BJ, Kassie F, Sasaki Y, Pfau W, Glatt H, Meinl W, Darroudi F, Knasmuller S (2004) Investigation of the genotoxic effects of 2-amino-9H-pyrido[2, 3-b]indole in different organs of rodents and in human derived cells. J Chromatogr B Analyt Technol Biomed Life Sci 802:167–173
21. Na HK, Surh YJ (2008) Modulation of Nrf2-mediated antioxidant and detoxifying enzyme induction by the green tea polyphenol EGCG. Food Chem Toxicol 46:1271–1278
22. Knasmuller S, Nersesyan A, Misik M, Gerner C, Mikulits W, Ehrlich V, Hoelzl C, Szakmary A, Wagner KH (2008) Use of conventional and -omics based methods for health claims of dietary antioxidants: a critical overview. Br J Nutr 99(E Suppl 1):ES3–ES52
23. Hoelzl C, Knasmuller S, Misik M, Collins A, Dusinska M, Nersesyan A (2009) Use of single cell gel electrophoresis assays for the detection of DNA-protective effects of dietary factors in humans: recent results and trends. Mutat Res 681:68–79
24. Tice RR, Agurell E, Anderson D, Burlinson B, Hartmann A, Kobayashi H, Miyamae Y, Rojas E, Ryu JC, Sasaki YF (2000) Single cell gel/comet assay: guidelines for in vitro and in vivo genetic toxicology testing. Environ Mol Mutagen 35:206–221
25. Gedik CM, Boyle SP, Wood SG, Vaughan NJ, Collins AR (2002) Oxidative stress in humans: validation of biomarkers of DNA damage. Carcinogenesis 23:1441–1446
26. Xu Y, Ho CT, Amin SG, Han C, Chung FL (1992) Inhibition of tobacco-specific nitrosamine-induced lung tumorigenesis in A/J mice by green tea and its major polyphenol as antioxidants. Cancer Res 52:3875–3879
27. Tamura K, Nakae D, Horiguchi K, Akai H, Kobayashi Y, Satoh H, Tsujiuchi T, Denda A, Konishi Y (1997) Inhibition by green tea extract of diethylnitrosamine-initiated but not choline-deficient, L-amino acid-defined diet-associated development of putative preneoplastic, glutathione *S*-transferase placental form-positive lesions in rat liver. Jpn J Cancer Res 88:356–362
28. Takabayashi F, Harada N, Tahara S, Kaneko T, Hara Y (1997) Effect of green tea catechins on the amount of 8-hydroxydeoxyguanosine (8-OHdG) in pancreatic and hepatic DNA after a single administration of *N*-nitrosobis(2-oxopropyl)amine (BOP). Pancreas 15:109–112
29. Inagake M, Yamane T, Kitao Y, Oya K, Matsumoto H, Kikuoka N, Nakatani H, Takahashi T, Nishimura H, Iwashima A (1995) Inhibition of 1, 2-dimethylhydrazine-induced oxidative DNA damage by green tea extract in rat. Jpn J Cancer Res 86: 1106–1111
30. Lodovici M, Casalini C, De Filippo C, Copeland E, Xu X, Clifford M, Dolara P (2000) Inhibition of 1, 2-dimethylhydrazine-induced oxidative DNA damage in rat colon mucosa by black tea complex polyphenols. Food Chem Toxicol 38:1085–1088
31. Giovannelli L, Testa G, De Filippo C, Cheynier V, Clifford MN, Dolara P (2000) Effect of complex polyphenols and tannins from red wine on DNA oxidative damage of rat colon mucosa in vivo. Eur J Nutr 39:207–212
32. Xue KX, Wang S, Ma GJ, Zhou P, Wu PQ, Zhang RF, Xu Z, Chen WS, Wang YQ (1992) Micronucleus formation in peripheral-blood lymphocytes from smokers and the influence of alcohol- and tea-drinking habits. Int J Cancer 50:702–705
33. Shim JS, Kang MH, Kim YH, Roh JK, Roberts C, Lee IP (1995) Chemopreventive effect of green tea (*Camellia sinensis*) among cigarette smokers. Cancer Epidemiol Biomarkers Prev 4:387–391
34. Glei M, Habermann N, Osswald K, Seidel C, Persin C, Jahreis G, Pool-Zobel BL (2005) Assessment of DNA damage and its modulation by dietary and genetic factors in smokers using the Comet assay: a biomarker model. Biomarkers 10:203–217
35. Balasubramanyam A, Sailaja N, Mahboob M, Rahman MF, Hussain SM, Grover P (2009) In vivo genotoxicity assessment of aluminium oxide nanomaterials in rat peripheral blood cells using the comet assay and micronucleus test. Mutagenesis 24:245–251
36. Speit G, Zeller J, Schmid O, Elhajouji A, Ma-Hock L, Neuss S (2009) Inhalation of formaldehyde does not induce systemic genotoxic effects in rats. Mutat Res 677:76–85
37. He P, Noda Y, Sugiyama K (2001) Green tea suppresses lipopolysaccharide-induced liver injury in d-galactosamine-sensitized rats. J Nutr 131:1560–1567
38. Abboud PA, Hake PW, Burroughs TJ, Odoms K, O'Connor M, Mangeshkar P, Wong HR, Zingarelli B (2008) Therapeutic effect of epigallocatechin-3-gallate in a mouse model of colitis. Eur J Pharmacol 579:411–417
39. de Maat MP, Pijl H, Kluft C, Princen HM (2000) Consumption of black and green tea had no effect on inflammation, haemostasis and endothelial markers in smoking healthy individuals. Eur J Clin Nutr 54:757–763
40. Hodgson JM, Puddey IB, Croft KD, Burke V, Mori TA, Caccetta RA, Beilin LJ (2000) Acute effects of ingestion of black and green tea on lipoprotein oxidation. Am J Clin Nutr 71:1103–1107
41. Princen HM, van Duyvenvoorde W, Buytenhek R, Blonk C, Tijburg LB, Langius JA, Meinders AE, Pijl H (1998) No effect of consumption of green and black tea on plasma lipid and antioxidant levels and on LDL oxidation in smokers. Arterioscler Thromb Vasc Biol 18:833–841
42. van het Hof KH, de Boer HS, Wiseman SA, Lien N, Westrate JA, Tijburg LB (1997) Consumption of green or black tea does not increase resistance of low-density lipoprotein to oxidation in humans. Am J Clin Nutr 66:1125–1132
43. Sung H, Min WK, Lee W, Chun S, Park H, Lee YW, Jang S, Lee DH (2005) The effects of green tea ingestion over four weeks on atherosclerotic markers. Ann Clin Biochem 42:292–297
44. Young JF, Dragstedt LO, Haraldsdottir J, Daneshvar B, Kall MA, Loft S, Nilsson L, Nielsen SE, Mayer B, Skibsted LH, Huynh-Ba T, Hermetter A, Sandstrom B (2002) Green tea extract only affects markers of oxidative status postprandially: lasting antioxidant effect of flavonoid-free diet. Br J Nutr 87:343–355
45. Dotan Y, Lichtenberg D, Pinchuk I (2004) Lipid peroxidation cannot be used as a universal criterion of oxidative stress. Prog Lipid Res 43:200–227
46. Kobayashi Y, Suzuki M, Satsu H, Arai S, Hara Y, Suzuki K, Miyamoto Y, Shimizu M (2000) Green tea polyphenols inhibit the sodium-dependent glucose transporter of intestinal epithelial cells by a competitive mechanism. J Agric Food Chem 48:5618–5623

47. Serrano J, Boada J, Ayala V, Gonzalo H, Jove M, Cacabelos D, Naudi A, Ilieva E, Pamplona R, Portero-Otin M, Delgado M, Espinel A (2008) Polyphenols antioxidant capacity: theory, practice and reality—in vitro, plasma and intracellular measurements of lipoxidative damage. In: COST B35 conference, lipid peroxidation associated disorders, development and validation of novel antioxidant substances for the prevention of lipid peroxidation. Kaunas, Lithuania
48. Shimizu M, Kobayashi Y, Suzuki M, Satsu H, Miyamoto Y (2000) Regulation of intestinal glucose transport by tea catechins. Biofactors 13:61–65
49. Glei M, Pool-Zobel BL (2006) The main catechin of green tea, (−)-epigallocatechin-3-gallate (EGCG), reduces bleomycin-induced DNA damage in human leucocytes. Toxicol Vitro 20: 295–300
50. Schwarz A, Maeda A, Gan D, Mammone T, Matsui MS, Schwarz T (2008) Green tea phenol extracts reduce UVB-induced DNA damage in human cells via interleukin-12. Photochem Photobiol 84:350–355
51. Collins AR (2004) The comet assay for DNA damage and repair: principles, applications, and limitations. Mol Biotechnol 26:249–261
52. Collins AR (1999) Oxidative DNA damage, antioxidants, and cancer. Bioessays 21:238–246
53. Valverde M, Rojas E (2009) The Comet assay in human biomonitoring. In: Dhawan A, Anderson D (eds) The Comet assay in toxicology. The Royal Society of Chemistry, Cambridge
54. Wu CD, Wei GX (2002) Tea as a functional food for oral health. Nutrition 18:443–444
55. Brown MD (1999) Green tea (*Camellia sinensis*) extract and its possible role in the prevention of cancer. Altern Med Rev 4:360–370
56. Fujiki H, Suganuma M, Imai K, Nakachi K (2002) Green tea: cancer preventive beverage and/or drug. Cancer Lett 188:9–13
57. Weisburger JH, Chung FL (2002) Mechanisms of chronic disease causation by nutritional factors and tobacco products and their prevention by tea polyphenols. Food Chem Toxicol 40:1145–1154