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Effects of a Brussels sprouts extract on oxidative DNA damage and metabolising enzymes in rat liver

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Abstract

The apparent anticarcinogenic effect of cruciferous vegetables found in numerous epidemiological and experimental studies has been associated with their influence on phase I and phase II metabolising enzymes as well as on the antioxidant status. In the present study we investigated the effect of administration of a Brussels sprouts extract on the expression at the mRNA level and/or catalytic activity in rat liver of three phase I enzymes [cytochrome P450-1A2 (CYP1A2),-2B1/2 (CYP2B1/2) and-2E1 (CYP2E1)] and two phase II enzyme [NADPH:quinone reductase (QR) and glutathione *S*-transferase pi 7 (GSTπ)], all previously suggested to be induced by vegetables. We also examined the activity and/or expression of several important antioxidant enzymes: glutathione peroxidase (GPx), catalase and γ-glutamyl-cysteine synthetase (GCS) and the activity of the repair enzyme 8-oxoguanine DNA glycosylase (OGG1). QR, GPx and catalase activity was also assessed in the kidneys. In order to examine a possible effect of the Brussels sprouts related to oxidative stress, we measured oxidative DNA damage in terms of 7-hydro-8-oxo-2′-deoxyguanosine (8-oxodG) and lipid peroxidation in terms of malondialdehyde (MDA) formation in the liver. Oral administration of an aqueous Brussels sprouts extract for 4 days was found to induce the expression of GST 1.3-fold ($P < 0.05$) and the activity of QR 2.6-fold in rat liver ($P < 0.05$). No significant differences were seen in the expression of the phase I enzymes. No differences in antioxidant enzyme activity/expression or OGG1 activity were observed. In a second experiment, administration of the Brussels sprouts extract for 3 or 7 days was found to increase the level of 8-oxodG in rat liver from 0.75 to 0.97 per 10^5 dG and from 0.81 to 0.97 per 10^5 dG, respectively ($P < 0.05$). No effects on MDA levels were found. The present results support the data obtained in several studies that consumption of cruciferous vegetables is capable of inducing various phase II enzyme systems. However, the observed increase in oxidative DNA damage raises the question of whether greatly increased ingestion of cruciferous vegetables is beneficial.

Keywords: Brussels sprouts; Oxidative DNA damage; 8-OxodG; Enzyme expression; CYP; GST

1. Introduction

The consumption of vegetables has been associated with a lowering in risk of several diseases, especially cancer (Steinmetz and Potter 1991; Block et al., 1992; Potter and Steinmetz 1996). Although the mechanisms underlying these effects are not fully understood, the potential of several substances in vegetables to act as antioxidants and scavenge reactive oxygen species (ROS) is thought to be important. ROS are continuously formed in all living cells as a consequence of various biochemical reactions and exposure to external factors. A balance normally exists in vivo between the production of ROS and the capacity of the endogenous antioxidant defence. Disturbance of this balance is termed oxidative stress, and results in cellular damage to cell constituents such as DNA, lipids and proteins (Halliwell and Aruoma, 1991).

Some vegetables have been found to have more pronounced anticarcinogenic effects than others. Cruciferous

Abbreviations: CYP, cytochrome P450; GCS hc, γ-glutamylcysteine synthetase heavy chain; GCS lc, γ-glutamylcysteine synthetase light chain; GPx, glutathione peroxidase; GSH, glutathione; GSTπ, glutathione *S*-transferase pi 7; MDA, malondialdehyde; OGG1, 8-oxoguanine DNA glycosylase; QR, NADPH:quinone reductase; 8-oxodG, 7-hydro-8-oxo-2′-deoxyguanosine; ROS, reactive oxygen species; TLC, thin-layer chromatography.

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vegetables such as Brussels sprouts, broccoli and cauliflower have in several animal experiments been shown to partly prevent chemical carcinogenesis and oxidative DNA damage when co-administered with a carcinogen (Verhoeven et al., 1997; Deng et al., 1998). In addition, epidemiological studies show a cancer preventive effect of cruciferous vegetables (Verhoeven et al., 1996). They are distinguished from other vegetables by their relatively high content of glucosinolates. Glucosinolates themselves exhibit low bioactivities, but on autolysis they are hydrolysed to release a number of products, mainly isothiocyanates and nitriles (Fenwick et al., 1983), which have been shown to have a chemopreventive effect (Verhoeven et al., 1997; London et al., 2000). A proposed mechanism is their influence on detoxification enzymes, since they have been found to induce both phase I enzyme activities (CYP) (Vang et al., 1990, 1991; Bjeldanes et al., 1991) and phase II enzymes (e.g. QR and GST) (Zhang et al., 1992). However, induction of CYP1A1 by one of the most potent hydrolysis products, indole-3-carbinol, has been associated with an increase in oxidative DNA damage in cell culture, which suggests that the induction can lead to leak of oxygen radicals (Park et al., 1996). It has also been found that induction of CYP1A may enhance bioactivation of a number of carcinogens, including benzo[*a*]pyrene and aflatoxin, although it may also serve as a detoxification pathway for other compounds. Furthermore, several studies have investigated possible harmful effects of treatment with cruciferous vegetables and different isothiocyanate species (Musk and Johnson, 1993; Musk et al., 1995; Kassie et al., 1996). One study found that crude *Brassica* juices caused genotoxic effects, chromosome aberrations and decrease in cell viability in vitro (Kassie et al., 1996). Also, high levels of Brussels sprouts in the diet of rats were found to cause, for example, enlargement of the liver and the kidneys, impaired renal function and microscopic hepatic changes (de Groot et al., 1991).

Additional possible chemoprotective effects of several glucosinolate derivatives are an increase of the endogenous antioxidant enzymes, as superoxide dismutase, GPx and catalase (Vang et al., 1995), an increase in enzymes associated with maintenance of glutathione (GSH) levels, for example the rate-limiting enzyme in the GSH synthesis GCS (Davis et al., 1993), and/or an increase in DNA repair enzymes, for example the OGG1 (Tsurudome et al., 1999).

In a previous study, oral administration of a Brussels sprouts extract was found to inhibit the 2-nitropropane-induced oxidative DNA damage, measured by 8-oxodG (Deng et al., 1998). In addition, Brussels sprouts appeared to decrease the background level of 8-oxodG in kidney and liver 6 h after the last administration, whereas the level was increased insignificantly by 14% in the liver 24 h after the last dose. The aim of the present study was to investigate the effect in rat liver of an aqueous extract of cooked Brussels sprouts on phase I and phase II enzymes, on the antioxidant enzymes GPx and catalase, on GSH synthesis enzyme GCS, and on DNA repair enzyme OGG1. In addition, the effect on oxidative damage to DNA in terms of 8-oxodG and lipids after administration in 3 or 7 days was examined.

2. Materials and methods

2.1. Chemicals

Trizma, potassium phosphate, cytochrome c, Triton X-100, NADH, menadione, 8-oxodG, dG, EDTA, glutathione, NADPH, glutathione reductase, *tert*-butyl hydroperoxide, H_2O_2, 1-methyl-2-phenylindol, glycerol, nuclease P1, sucrose, HEPES, KCl, DTT, ethidium bromide, agarose, acrylamide/bis-acrylamide 19:1 stock solution, TEMED, ammoniumpersulfate, bromophenol blue and xylene cyanol dye solution, TBE powder, ammonium acetate and urea were all obtained from Sigma (St Louis, MO, USA); 1,1,3,3-tetramethoxypropane was from Aldrich (Milwaukee, USA); NaCl, EDTA, HCl, mannitol, H_3PO_4 85% and $NaClO_4$ were from Riedel-deHäen (Seelze, Germany); NaOH, NaAc (sodium acetate), $ZnCl_2$, SDS, $C_5H_{12}O$ (isoamylalcohol), 2-nitropropane, pepstatin A, chymostatin, antipain dihydrochlorid, leupeptin, triethylammonium acetate and formamide were from Merck (Darmstadt, Germany); T4-polynucleotid kinase and 1× kinase reaction buffer was from Boehringer Mannheim (Hørsholm, Denmark); Coomasie® Protein Assay Reagent was from Pierce (Rockford, USA); alkaline phosphatase and HEPES were from Boehringer Mannheim (Mannheim, Germany); and chloroform and acetonitrile were from Romil (Cambridge, UK); oligonucleotides were obtained from DNA Technology (Århus, Denmark); piperidine was from Struers KEBO Lab A/S (Albertslund, Denmark) and Redivue™ [γ-^{32}P]dATP was from Amersham Pharmacia Biotech (Hørsholm, Denmark).

2.1.1. Brussels sprouts extract

Fresh Brussels sprouts were obtained from an ecological grower in Odsherred, Denmark, and washed with tap water and subsequently with distilled water and stored at −80°C. 1 kg Brussels sprouts was thawed and ground in a home-mixer with 40 ml distilled water. The resultant juice and residues were cooked together in a microwave oven for 3 min and squeezed through gauze. The residues were repeatedly washed with distilled water until the colour changed from green to white. The combined solution of cooked juice and water extract from the residues was freeze-dried and subsequently stored at 4°C. The powder was resuspended in distilled water just before use, to yield an extract. This procedure

was performed twice, resulting in 52.25 g powder/kg fresh Brussels sprouts, which was used in the first study and 62.22 g powder/kg fresh Brussels sprouts, which was used in the second study. Brussels sprouts are particularly rich in the glucosinolates sinigrin and glucobrassicin (Sones et al., 1984). In agreement, we have previously analysed Brussels sprouts extract identical to the present. With thin-layer chromatography (TLC) we found major spots corresponding to the glucosinolates sinigrin and gluconapin, and smaller spots corresponding to isothiocyanates, allyl isothiocyanates, phenyl isothiocyanates. In HPLC, seven major peaks were found with one major peak corresponding to sinigrin (Zhu et al., 2000).

2.2. Animals and treatment

Male Wistar rats, approximately 6 weeks old and 180–250 g body weight, were used. The animals received standard laboratory diet and tap water ad lib. They were housed four to a cage with aspen wood bedding (Finntapwei, Finland) in an environmentally controlled animal facility operating on a 12-h dark/light cycle and 55% humidity.

Two separate studies were conducted. In order to study the effects on enzymes, 16 rats were randomly allocated in a control group and a treatment group with eight rats in each. The treatment group received the Brussels sprouts extractly corresponding to 7 g fresh vegetables by gavage for 4 days. The animals were killed by decapitation 6 h after last dose extract/vehicle. The livers and kidneys were dissected and immediately frozen in liquid nitrogen and stored at −80°C until use. In the second study, 24 rats were randomly allocated in four groups with six rats in each group. Two groups received Brussels sprouts extract corresponding to 6.4 g fresh vegetable and two groups received corresponding vehicle, for 3 and 7 days, respectively. The animals were killed by decapitation 24 h after the last treatment and the liver was secured as above.

2.3. Enzyme activities

2.3.1. Catalase, QR and GPx

The livers and the kidneys were homogenised on ice in 20 volumes of 0.1 M potassium phosphate buffer, pH 7.4. The homogenates were first centrifuged at 9000 *g* for 20 min and the supernatants were then centrifuged at 100,000 *g* for 1 h. The resultant supernatants, termed the cytosolic fractions, were stored at −80°C until use. All operations were carried out at 4°C. The enzyme activities were all measured spectrophotometrically. Catalase activity was measured using hydrogen peroxide as substrate (Aebi, 1984), QR activity was measured using menadione as substrate for QR followed by reduction of cytochrome C (Segura-Aguilar et al., 1992), and GPx activity was determined using GSH as cofactor and hydrogen peroxide as substrate, followed by oxidation of NADPH (Paglia and Valentine, 1967). Protein concentration was determined using a commercially available method kit (Pierce, Rockford, IL, USA). The enzyme activities are expressed as activity per mg organ protein.

2.3.2. OGG1

The livers were homogenised on ice in three volumes of cold buffer (20 mM HEPES pH 7.9, 75 mM KCl, 5 mM dithiothreitol, 5 mM EDTA, 10% glycerol, 5 μg/ml pepstatin A, 5 μg/ml chymostatin, 5 μg/ml antipain dihydrochloride, 5 μg/ml leupeptin hydrogensulfate) and centrifuged at 10,000 *g* for 40 min at 4°C to obtain the crude extract. The supernatants were stored at −80°C until use. The supernatants (25 μg protein) were incubated at 37°C for 30 min with 0.5 pmol ^{32}P-labeled 8-oxodG containing oligonucleotide (5′-GGC GGC ATG ACC CG^{oxo}G AGG CCC ATG-3′) (DNA Technologies, Aarhus, Denmark) and 10 pmol cold undamaged oligonucleotide (3′-CCG CCG TAC TGG GCC TCC GGG TAG-5′) (DNA Technologies) in a reaction mixture containing 20 mM HEPES pH 7.9, 75 mM KCl, 5 mM dithiothreitol, 5 mM EDTA, 10% glycerol in a final volume of 20 μl. Oligonucleotides were then precipitated by addition of ammonium acetate to a concentration of 2 M and 2.5 volume of 96% cold ethanol. The DNA was pelleted, dried and resuspended in 10 μl formamide loading buffer (80% formamide, 1 mM EDTA, 0.05% xylenecyanol green and 0.05% bromophenol blue). The samples were electrophoresed on a denaturing 20% polyacrylamide/7 M urea/TBE gel in 1×TBE for 1 h at 15 W and subjected to autoradiography on a PhosphorImager screen (Imager Plate BSAIII™), developed in a Fujix Bio-Imaging Analyser System BAS2000™. Quantification of the results was performed by computer image processing using the TINA 2.08 software (©1993 Isotopenmeßgeräte GmbH). The repair activity was expressed as the ratio of the radioactivity of the excised fragment to the total radioactivity (uncleaved DNA + cleaved DNA).

2.4. Enzyme expression

The following cDNA probes were used: CYP1A2 (Kimura et al., 1984), CYP2B1/2 (Adesnik et al., 1981), CYP2E1 (Song et al., 1986), GSTπ (Suguoka et al., 1985), GCS light chain (GCSlc) (Huang et al., 1998) and heavy chain (GCShc) (Mulcahy et al., 1997). Their specificity was ascertained by autoradiography of Northern blots, showing signals from hybridised mRNA at the expected position in relation to rRNA 18S and 28S. DNA fragments (inserts) cut by restriction enzymes purchased from Promega (San Luis Obispo, CA, USA) and Boehringer (Mannheim, Germany), were separated by agarose gel electrophoresis, excised from the gel

under UV light, and isolated by Wizard® DNA Clean-Up System (Promega). The probes were labelled by rediprime labelling kit (RPN 1633/1634, Amersham, Buckinghamshire, UK) and isolated by NucTrap® purification columns (Stratagene, La Jolla, CA, USA).

Total RNA was extracted from 200 mg frozen liver tissue using a commercially available kit (Rneasy® midi kit, Qiagen, Bothell, WA, USA). For slot blot analysis, nylon membranes were incubated in bi-distilled H_2O for 10 min, then in 10× SSC (standard saline citrate) for 10 min, and placed in a Schleicher & Schuell Minifold®. 10× SSC was loaded into the slots and vacuum applied until all the slots were empty. To each slot 10× SSC was added, followed by 5 μg RNA of a sample diluted to 50 μl with TE buffer (Tris–Cl pH 8.0 10 mM, EDTA 1 mM, 20× SSC), mixed with 30 μl of 20× SSC and formaldehyde 37% 20 μl, incubated in 15 min at 65°C, placed on ice and diluted by 300 μl SSC 10× before loading. After emptying the slots by vacuum, 400 μl 10× SSC was added and vacuum applied. The membrane was placed in a hybridisation vessel (Hybaid 150×35 mm), washed with 25 ml of deionised water, then 10 ml QuickHyp® (Stratagene) was added and the membrane prehybridised for 30 min at 68°C. The probe and 100 μl salmon sperm DNA were mixed and boiled for 5 min, 500 μl QuickHyp® was added, mixed and placed in the vessel for hybridisation at 68°C for 1 h. The filters were washed twice for 15 min at room temperature by 2×SSC and 0.1% SDS and for 30 min at 60°C by 0.1×SSC and 0.1% SDS. Autoradiography was made on an imaging plate BASIII and the hybridisation signal was analysed in a FUJIX bioimaging analyser system BAS 2000 (FUJI Photo Film Co.). Afterwards the filters were immersed in boiling SDS 0.1% and glycerol 1%, left to cool and washed in sterile water, and then hybridised with cDNA for 18S rRNA and read as above, used to adjust for variations in RNA loading.

2.5. Malondialdehyde

The livers were homogenised in ice-cold 20 mM Tris–HCl buffer, pH 7.4 (0.1 g tissue/ml) and then centrifuged at 3000 *g* for 10 min. The supernatants were stored at −80°C until use. MDA, a product of lipid peroxidation, was measured spectrophotometrically at 586 nm after reaction with 1-methyl-2-phenylindole (Esterbauer et al., 1991). The supernatant (400 μl diluted 1:10) was added to 1.3 ml 10.3 mM 1-methyl-2-phenylindole diluted 3:4 in methanol. MDA is expressed as concentration per mg protein.

2.6. 8-oxodG analysis

The livers and the lungs (600 mg) were homogenised in 30 ml HEPES buffer (250 mM mannitol, 70 mM sucrose, 5 mM HEPES, pH 7.4). The homogenates were then centrifuged at 1000 *g* in 10 min. The pellet was resuspended in 600 μl saline. All the operations were carried out at 4°C. The DNA was extracted, precipitated and digested as previously described (Deng et al., 1998). The 8-oxodG/10^5 dG ratio was measured using a HPLC system with electrochemical and UV detection as previously described (Loft and Poulsen, 1999).

2.7. Statistics

The groups were compared by means of analysis of variance (ANOVA), followed if significant by a least significant difference test (LSD-test) for identification of differences between the groups. Some variables were log transformed in order to obtain a normal distribution before analysis. Probability values less than 0.05 were considered statistically significant.

3. Results

3.1. Enzyme expression and activity

Table 1 shows the results of the Brussels sprouts treatment on the activities of QR, GPx, catalase and OGG1. A significant 2.6-fold increase in QR activity in the liver and a small non-significant increase in the kidney were found following Brussels sprouts treatment. No significant changes in the activity of GPx, catalase and OGG1 could be detected in the liver or the kidney.

The expression levels of the metabolising enzymes and GCS measured after 4 days of treatment with Brussels

Table 1
Activities of QR, GPx, catalase and OGG1 following administration of Brussels sprouts extract for 4 days[a]

	Control	Brussels sprouts (7 mg/kg for 4 days)
QR activity (μmol/min/mg protein)		
Liver	87±41	222±114*
Kidney	48±4	60±15
GPx activity (mU/mg protein)		
Liver	819±200	905±117
Kidney	578±91	570±53
Catalase activity (U/mg protein)		
Liver	1160±175	1135±86
Kidney	332±110	271±64
OGG1 activity (% incision activity)		
Liver	19.8±1.6	20.6±6.8

[a] QR, NADPH: quinone reductase; GPx, glutathione peroxidase; OGG1, 8-oxoguanine DNA glycosylase.

*$P < 0.05$ vs corresponding control.

sprouts are shown in Fig. 1. A significant increase (1.3-fold) was found in the expression level of the phase II enzyme GSTπ. No significant changes were found in the expression levels of CYP1A2, CYP2B1/2, CYP2E1, GCSlc or GCShc, although a small increase was observed in the expression level of CYP1A2 (15.9%) and a small decrease was seen in the expression level of GCSlc (17.7%).

3.2. Oxidative damage

Fig. 2 shows the effects of Brussels sprouts treatment on oxidative damage to DNA measured by the level of 8-oxodG in rat liver DNA. Significant 1.3- and 1.2-fold increases in the 8-oxodG level were found following 3 ($P < 0.002$) and 7 ($P < 0.02$) days of Brussels sprouts treatment. No changes in lipid peroxidation measured by MDA in the liver could be distinguished, neither after 3 days; 0.21±0.08 μM MDA/mg protein compared to the control 0.19±0.03 μM MDA/mg protein, nor after 7 days of treatment; 0.19±0.03 μM MDA/mg protein compared to the control 0.19±0.02 μM MDA/mg protein.

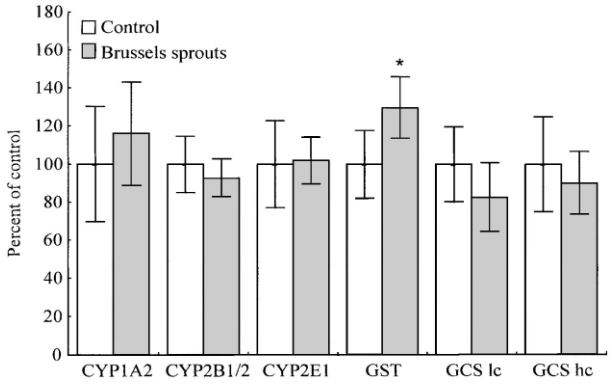

Fig. 1. Effects of Brussels sprouts treatment in 4 days on expression levels of CYP1A2, CYP2B1/2, CYP2E1, GST, GCSlc and GCShc. Each group consisted of eight rats. *$P < 0.05$ vs corresponding control. CYP1A2: cytochrome P450 1A2, CYP2B1/2: cytochrome P450 2B1/2, CYP2E1: cytochrome P450 2E1, GST: glutathione *S*-transferase, GCSlc: γ-glutamylcysteine synthase light chain, GCShc: γ-glutamylcysteine synthase, heavy chain.

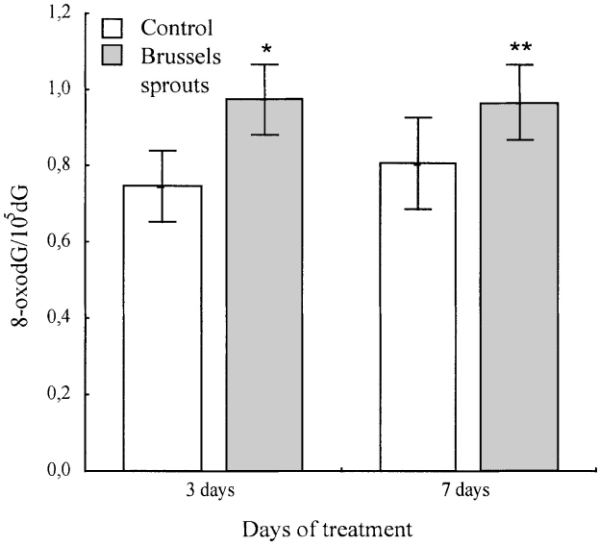

Fig. 2. Effects of Brussels sprouts treatment in 3 and 7 days on 8-oxodG levels in rat livers. The number of animals in each subgroup varied from five to six. The 8-oxodG levels are given as mean with S.D. * $P < 0.002$ vs corresponding control; **$P < 0.02$ vs corresponding control. 8-oxodG; 7-hydro-8-oxo-2′-deoxyguanosine.

4. Discussion

Brussels sprouts treatment was found to significantly increase the activity of QR and the expression of GST in rat liver, whereas no effect was observed on the activity of GPx, catalase and OGG1 or on the expression of CYP1A2, CYP2B1/2, CYP2E1, GCSlc or GCShc. In addition, Brussels sprouts treatment was found to increase oxidative DNA damage in rat liver whereas no changes in lipid peroxidation were found.

Previous studies have found that cruciferous vegetables have an effect on the activity level and expression level of various metabolism enzymes (Wortelboer et al., 1992; Staack et al., 1998; Cashman et al., 1999; Lampe et al., 2000) and antioxidant enzymes in rodents and humans (Vang et al., 1995, 1997). In the present study, no significant changes were found in the CYP expression levels, although the expression level of CYP1A2 increased 15.9% (Fig. 1). Several other groups have investigated the effect of cruciferous vegetables on phase I enzymes (Vang et al., 1990, 1991; Wortelboer et al., 1992; Manson et al., 1997; Lampe et al., 2000). In most studies, CYP1A1/2 and CYP2B1/2 expression and activity are increased after administration of cruciferous vegetables, and this effect has been found to be associated with the content of different species of glucosinolate hydrolysis products, especially indole compounds (Vang et al., 1990; Wortelboer et al., 1992; Manson et al., 1997). One reason why we could not distinguish any effects on the CYP enzymes as a result of Brussels sprouts ingestion could be due to differences in the glucosinolate composition that exist between different *Brassica* species (Tiedink et al., 1988). Previous analysis of an extract similar to the present by TLC and HPLC indicated that sinigrin and gluconapin and the corresponding isothiocyanates were the major components in agreement with the usual content of Brussels sprouts (Sones et al., 1984; Zhu et al., 2000).

The effect of the Brussels sprout extract on phase II enzymes in terms of increased expression of GST and activity of QR in the liver are comparable to the results obtained by other groups as summarised by Verhoeven et al. (1997). It seems doubtful, however, that this increase in phase II enzymes should be the only responsible factor for the previously reported anticarcinogenic effect

of *Brassica* vegetables. Also, epidemiological studies find that people with deletions in GSTM1 and/or GSTT1 have a higher protective effect of isothiocyanates in relation to lung cancer and colorectal cancer, compared to men with the enzyme (Lin et al., 1998; London et al., 2000). This is explained by the fact that the absence of GST decreases the elimination of isothiocyanates, which points to the presence of other more impotent anti-carcinogenic properties of the isothiocyanates.

Cruciferous vegetables are also known for their antioxidant properties, and we examined the effect of the extract on the antioxidant enzymes GPx, catalase and GCS as well as on the activity of OGG1, a repair enzyme which recognises the oxidative damaged base 8-oxoguanine. We found no changes in the activities of GPx, catalase and OGG1 or in the expression of GCS, suggesting that the reported antioxidant effects are not due to an induction of the endogenous antioxidant system or an increase in oxidative DNA repair. However, other groups have investigated the effects of cruciferous vegetables or glucosinolate derivatives on antioxidant enzymes (Davis et al., 1993; Vang et al., 1995, 1997). In two of these experiments, GPx activity was measured in rat liver and kidney following treatment with freeze-dried broccoli corresponding to 12 g of fresh broccoli per day for 7 days. In the first study, a significant increase in renal GPx activity was found, which was correlated with the content of glucosinolates (glucoerucin and glucobrassicin). No effect could be recognised in the liver. In the second study, no effect in liver or kidney was found. The difference between the two experiments was explained by differences in the level of glucosinolate content in the dried broccoli powder. Another study found that cyanohydroxybutene, a glucosinolate derivative also present in Brussels sprouts, increased GCS expression but not activity in rat liver (Davis et al., 1993). These results indicate that cruciferous vegetables, dependent of the composition and concentration of glucosinolates, could have an effect on the endogenous antioxidant system. In addition, Brussels sprouts constituents, including glucosinolates may have direct antioxidant effects. Thus, an extract similar to that used in the present study was shown to protect isolated calf thymus DNA from 8-oxodG generation by a Fenton reaction and various forms of UV light (Zhu et al., 2000).

In the second experiment of this study, the treatment with Brussels sprouts extract for 3 and 7 days caused a significant increase in oxidative DNA damage in the liver, measured by 8-oxodG, whereas we did not find any significant changes in lipid peroxidation in the liver. In a previous experiment performed in the laboratory (Deng et al., 1998), a Brussels sprouts extract similar to the present was administrated for 4 days, after which oxidative DNA damage was measured in the liver, the kidneys and the bone marrow. A non-significant decline in 8-oxodG was observed 6 h after the last Brussels sprouts treatment in the kidney and the liver. 24 h after the last administration a non-significant increase in 8-oxodG was seen in the liver and the bone marrow, whereas there was a significant decrease in the renal 8-oxodG. In addition, a significant decline in urinary 8-oxodG excretion was observed in the 24 h following the last Brussels sprouts treatment. Moreover, pretreatment with Brussels sprouts extract prevented substantial increases of 8-oxodG formation as well as urinary 8-oxodG excretion in liver, kidney and bone marrow induced by to 2-NP administration.

Two studies investigating the effect of Brussels sprouts in human diet have been conducted (Verhagen et al., 1995, 1997). In the first study, a significant decrease in 8-oxodG excretion was found in five men, after 3 weeks of consuming 300 g cooked Brussels sprouts per day. In a second study, a decrease in 8-oxodG in four of five men and in two of five women following 3 weeks of consuming 300 g cooked Brussels sprouts was found.

Comparing these data implies that cooked Brussels sprouts decreases 8-oxodG concentrations in the urinary excretion, which expresses total body DNA damage, although this effect has only been demonstrated in men. However, further investigation of oxidative DNA damage in specific rat organs shows that the treatment with Brussels sprouts may result in both a decrease and an increase, depending on the time of measurement, the concomitant administration of potent damage inducing agent and the organ measured. Even though the overall effect can be a decrease in 8-oxodG levels, the increase at some time points raises the question of whether extreme intake of Brussels sprouts is recommendable.

Several investigations have been conducted to examine the possible harmful effects of Brussels sprouts treatment. One study tested the effects of crude juices from *Brassica* vegetables in vitro (Kassie et al., 1996). In a bacterial assay they found that all the juices caused genotoxic effects, the most toxic being Brussels sprouts. They also examined mammalian cells, and found that the juices induced structural chromosome aberrations (CA) and caused a decrease in cell viability (again Brussels sprouts as the most potent). This genotoxic effect has been shown to be associated with different isothiocyanate species (Musk and Johnson, 1993; Musk et al., 1995; Kassie and Knasmuller, 2000). The CA test has recently been subjected to some criticism, since positive results are obtained more often in this test than in other genotoxic tests (Muller and Sofuni, 2000). However, another study investigated the effects of high levels of Brussels sprouts in the diets of rats (de Groot et al., 1991). They found enlargements of liver and kidneys, impaired renal function, increased prothrombin time indicating retardation in the process of blood coagulation and microscopic hepatic changes indicative of liver damage.

In addition to the harmful effects, carcinogenicity studies on animals have shown that cruciferous vegetables or glucosinolates/isothiocyanates inhibit the carcinogenic effects of several environmental toxins (Stoewsand et al., 1988; Zhang and Talalay, 1994). In most of these studies, relatively large doses of model carcinogen were administrated after or concomitant with the vegetables or glucosinolates/isothiocyanates.

In summary, it seems that cruciferous vegetables, including Brussels sprouts, exhibit both beneficial and harmful effects. Possible explanations could be related to differences in the composition and concentration of the glucosinolates/isothiocyanates consumed, and/or the quantity of cruciferous vegetables in the diet.

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