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Induction of Hepatic Glutathione S-transferase Activity in Mice Administered with Various Vegetable Extracts

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Abstract

The effect of various vegetables commonly consumed by Koreans on the induction of glutathione S-transferase(GST) activity in mice was assessed. The extract of vegetable dissolved in propylene glycol(5ml/kg body wt.) was administered to ICR female mice 6 to 8 weeks old via gavage during 5 days. The changes of body weight and liver weight of all treated groups were not significantly different compared with control group. Hepatic protein contents of treated groups compared with control group were not significantly different except BHT treated group. The induction of GST activity in liver cytosol of mice was the greatest with broccoli, followed by radish, wild green onion, turnip, and green onion. The induction of GST activity in liver cytosol increased up to 1.5 to 1.8-folds at a dose of 24g fresh vegetable/mouse. The induction of combination between vegetables was the highest with the combination of broccoli and radish(1.83-fold), followed by that of broccoli and green onion(1.72-fold), and that of broccoli and turnip(1.50-fold).

Key words: glutathione S-transferase, induction, vegetables, mice

INTRODUCTION

Consumption of cruciferous vegetables has been associated with a reduction in the incidence of cancer at several sites in human body(1,2). Feeding of crucifers is known to induce enzymes responsible for xenobiotic metabolism, and thereby accelerate the metabolic disposal of xenobiotics(3-5). Especially, induction of phase II detoxification enzymes, such as glutathione S-transferase(EC 2.5.1.18) and quinone reductase [(NAD(P)H : (quinone : acceptor) oxidoreductase, EC 1.6.99.2] in rodent tissue affords protection against carcinogens and other toxic electrophiles(3,6). Isothiocyanates of cruciferous vegetables are monofunctional inducers of phase II enzymes(7-10). Recently, chemoprotective effect of cruciferous vegetables such as broccoli(11), cabbage(12,13), cauliflower(14), and Brussels sprout(15), based on the induction of the activities of xenobiotic-metabolizing enzymes in animals, was assessed. However, there are only a few reports on the comparison of GST-induction by vegetables. Moreover, the induction effect of vegetables commonly used in Koreans was not examined.

In this study, the GST-induction by commonly consumed cruciferous vegetables, which were harvested in Korea, and their combinations, were assessed.

MATERIALS AND METHODS

Materials

Fresh cruciferous vegetables(cultivar unknown) were purchased from local market, Taejon, Korea. Dichloromethane were purchased from Merk Chemical Co.(Germany). 1-Chloro-2,4-dinitrobenzene(CDNB), propylene glycol, bovine serum albumin and glutathione were products of Sigma Chemical Co. Standard sulforaphane was purchased from LKT Labs, Inc.(St.Paul, MN, USA).

Preparation of vegetable extracts

Samples(300g or 600g) of vegetable were coarsely cut, and then blended in a Waring Blendor(Waring Products, New Hartford, CT) containing 600 or 1,200ml distilled water at the highest speed for 2min. Each homogenate was filtered through several layers of cheese cloth, and the filtrate from each homogenate was extracted with 50ml dichloromethane. The dichloromethane layer was recovered, and centrifuged(2,000×g, 10min). Dichloromethane extract of vegetable was evaporated using a rotary evaporator

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(<40°C), and then the residue was dissolved in propylene glycol(PG). The sample was filtered through 0.45μm porosity organic solvent-resistant filters before use.

GC/MS analysis for volatile sulfur compounds of vegetables

The concentrated dichloromethane extract of vegetable was dissolved in 100μl of dichloromethane, then 1μl of extract was injected into GC/MSD. Quantitative analysis of sulforaphane was analyzed by selected ion monitoring mode with standard sulforaphane and reported by Kim et al.(16). Other sulfur compounds such as isothiocyanates and sulfides were identified by mass spectrum. The condition of GC/MSD was showed in Table 1.

Animals

Female ICR mice(mean weight±SE ; 22.5±2.5g) at 6 to 8 weeks of age were used in this experiment. They were housed in polycarbonate cage(five per cage) and were fed unrestricted amounts of water and pelleted commercial diet(SamYang Co. Korea). The temperature and the relative humidity were 23±3°C and 60±10%, respectively, and 12hr light/dark cycles were maintained. All animal experiments were in compliance with National Institutes of Health Guidelines(17). The mice which were assigned randomly to treated groups were weighed individually every day. Each mouse received by gavage either 5ml/kg wt of polypropylene glycol(PG), or vegetable extract(12 or 24g fresh vegetable/mouse) in PG daily for 5 days, and then animals were fasted for 1 day before the sacrifice by cervical dislocation.

Preparation of liver homogenates and tissue fractions

All procedures were carried at 0~4°C under sterile conditions. Livers were quickly removed, weighed, rinsed with 0.15M KCl in 2mM EDTA(pH 7.0), and homogenized in three volume of 0.25M sucrose(3ml/g) solution using a tissue homogenizer with a teflon pestle. The homogenate was centrifuged at 12,000×g for 20min, and the supernatant was further centrifuged at 100,000×g for 60 min to yield a microsomal pellet and cytosol supernatant. Cytosol supernatant was stored in 1.5ml aliquot at -80°C, and microsomal fraction, resuspended in 0.05M Tris-HCl buffer(pH 7.4), stored in 1.5ml aliquot at -80°C.

Table 1. The condition for volatile sulfur compounds by GC/MSD

Gas chromatograph : HP 5890 II
Mass selective detector : HP MSD 5972
Column : HP 5MS capillary direct column(0.27mm×30m)
Temperature : Column : 60~230°C(10°C/min)
Detector : 250°C
Injector : 250°C
Carrier gas : Helium(0.8ml/min, 5.6psi)

Enzyme assays

Protein was determined by the method of Bradford(18) using bovine serum albumin as a standard. Cytosolic GST activity was determined by the method of Habig et al.(6). The change in absorbance at 340nm was monitored 3 min at 37°C using spectrophotometer(Milton Roy 1201) with extinction coefficient. 9.6/mM.cm. Enzyme activities were expressed as the mean±S.D. of four determinations using 1-chloro-2,4-dinitrobenzene as substrate in the presence of 0.1mM glutathione.

Statistical analyses

All statistical analyses were performed on a SAS program. Duncan's multiple range test was used to determine significant difference among treatment groups after initial demonstration of a treatment-related effect by analysis of variance(19).

RESULTS

The effect of vegetable extracts on body weight and organ weight of animals was demonstrated in Table 2. All mice treated with vegetable extract survived gavage administration of 12g fresh vegetable/mouse for 5 days, while only one of five mice given a high dose of BHT (5g/kg) survived. In general, the body weight decreased by about 7 to 15%, 5 days after the start of treatment with various vegetable extracts. Compared with the vehicle-treated control, no significant loss of body weight was observed with any vegetable extract, and behavior and food intake were not significantly altered at doses of vegetable extracts used. When the change of liver weight from mice administered with vegetable extracts was examined, it was found that there was no remarkable difference of liver weight after the administration of vegetable extracts except garlic extract, which expressed an increase of approximately 20%, compared with vehicle-treated control group.

Subsequently, the change of hepatic GST activity af-

Table 2. Body weight, change of body weight, liver weight and liver protein content of ICR mice liver cytosol administered with various vegetable extracts

Treatment	Body weight(g)	Change of body weight	Liver weight (g/100g)	Liver protein (μg/ml)
Experiment 1				
Control	23.32±0.86$^{ab,2)}$	0.14±0.21$^{4)NS}$	0.18±0.13^{NS}	16.28±2.98^{NS}
Propylene glycol	21.89±0.85$^{bc,1)}$	-0.04±0.30	1.15±0.07	17.46±3.80
Radish$^{1)}$	22.69±1.96^{ab}	0.32±0.28	1.22±0.15	18.97±1.70
BHT$^{3)}$	24.40±0.00^{a}	0.00	1.23±0.00	12.97±0.00
Experiment 2				
Control	23.37±2.41$^{N.S}$	-1.98±0.83^{ab}	0.98±0.10^{ab}	10.05±2.40^{c}
Propylene glycol	22.43±1.88	-2.78±0.56^{bc}	0.87±0.07^{bc}	12.09±1.39^{bc}
Red cabbage	21.90±1.34	-2.74±0.56^{bc}	0.85±0.06^{c}	11.51±1.51^{bc}
Wild green onion	23.57±1.34	-2.86±0.69^{bc}	1.00±0.13^{a}	15.57±3.08^{a}
Broccoli	26.27±2.41	-2.88±0.51^{bc}	1.11±0.14^{bc}	11.20±0.72^{abc}
Green onion	22.94±1.15	-2.44±0.36^{bc}	0.93±0.07^{abc}	15.73±1.63^{a}
Mustard leaf(Dolsan)	22.62±2.18	-2.82±0.40^{bc}	0.90±0.07^{abc}	14.06±2.64^{ab}
White cabbage	22.54±1.04	-2.84±0.73^{bc}	0.87±0.05^{bc}	10.62±2.46^{c}
Leek	23.13±1.31	-1.36±0.70^{a}	0.96±0.04^{abc}	14.11±3.00^{ab}
Radish seed sprout	23.14±1.34	-3.02±0.66^{c}	0.90±0.04^{abc}	14.51±1.80^{ab}
Experiment 3				
Control	25.72±2.36$^{N.S}$	-3.62±1.53$^{N.S}$	1.01±0.06$^{N.S}$	11.74±2.56^{a}
Propylene glycol	26.47±2.48	-3.84±1.52	1.14±0.06	11.02±2.55^{a}
Broccoli	26.27±2.41	-2.88±0.51	1.11±0.14	11.20±0.72^{a}
Turnip	25.33±1.18	-3.52±0.47	1.03±0.06	10.00±1.28^{b}
Green onion	23.36±1.46	-3.46±1.26	1.08±0.11	11.28±1.22^{a}
Radish leaf	25.23±1.48	-3.54±1.18	1.04±0.11	11.12±1.86^{a}
Experiment 4				
Control	29.29±3.06^{ab}	-3.58±1.14^{a}	1.10±0.14^{b}	15.65±1.39^{ab}
Propylene glycol	29.01±3.32^{ab}	-4.24±1.37^{ab}	1.11±0.09^{b}	13.43±0.66^{b}
Garlic	31.82±1.24^{a}	-5.26±0.89^{b}	1.44±0.21^{a}	16.48±1.64^{a}
Radish skin	24.46±6.97^{b}	-4.32±0.50^{ab}	1.07±0.11^{b}	17.08±2.64^{a}
Kale	28.63±2.61^{ab}	-3.44±0.76^{a}	1.09±0.09^{b}	16.61±3.95^{a}
Dried radish leaf	28.20±2.80^{ab}	-3.40±0.58^{a}	1.13±0.16^{b}	16.97±0.85^{a}

[1)]Solvent extract of fresh vegetables(12g/mouse), garlic(6g/mouse) was administered to 6 to 8-week-old female ICR mice by gavage a daily dose(5ml/kg weight) for 5 days
[2)]Mean±SD
[3)]BHT(1g/kg) dissolved in corn oil(5ml/kg)
[4)]Any two means in the same row with different superscript are significantly different($p<0.05$)
[N.S.]Not significantly different

ter the gavage administration of 12g fresh vegetable/mouse was assessed(Table 3). The increase(15~58%) in hepatic GST activity was shown by vegetable extracts, compared to vehicle-treated control; a modest increase(15~27%) by mustard leaf extract(1.27-fold), kale extract(1.26-fold), dried radish leaf extract(1.17-fold) and radish seed sprout extract(1.15-fold); an apparent increase(35~47%) by green onion extract(1.47-fold), wild green onion extract (1.46-fold), leek extract(1.41-fold), red cabbage extract (1.40-fold), white cabbage extract(1.35-fold) and radish leaf extract(1.40-fold); a remarkable increase(50-58%) by broccoli extract(1.57-fold), garlic extract(1.58-fold) and turnip extract(1.50-fold). Although the highest effect was expressed by broccoli, a Western vegetable, it is noteworthy that commonly-consumed Korean vegetables such as green onion, radish or leek showed apparent increase in hepatic GST activity

When the induction of hepatic GST by vegetable extract at different doses was assessed(Table 4), broccoli extract(1.67-fold) and radish extract(1.72-fold) at 24g fresh vegetable/mouse increased the induction of GST activity by 10% and 12.5%, respectively, compared with the induction by a dose of 12g fresh vegetable/mouse. Also, the induction effect of green onion extract differed greatly according to the dose. That is, administration of 24g fresh vegetable/mouse showed 1.74-fold, while an increase of 1.5-fold was observed when 12g fresh vegetable/mouse was intubated.

Table 3. **Hepatic glutathione S-transferase activity in ICR mice administered with various vegetable extracts**

Treatment	GST activity (nmol/min/mg protein)	Ratio to vehicle-treated control
Experiment 1		
Control	134.0± 6.6$^{c,2)}$	0.96±0.05^{c}
Propylene glycol	140.0± 9.5$^{c,4)}$	1.00±0.07^{c}
Radish[1)]	217.2± 28.7^{b}	1.55±0.21^{b}
BHT[3)]	828.0± 0.0^{a}	5.91±0.21^{a}
Experiment 2		
Control	192.0± 11.4^{c}	0.92±0.05^{c}
Propylene glycol	209.8± 9.4^{bc}	1.00±0.05^{bc}
Red cabbage	296.2± 55.0^{ab}	1.41±0.26^{ab}
Wild green onion	306.1± 89.4^{a}	1.46±0.43^{a}
Broccoli	292.6± 76.0^{abc}	1.40±0.36^{abc}
Green onion	273.8± 36.8^{abc}	1.31±0.18^{abc}
Mustard leaf(Dolsan)	266.4± 29.8^{abc}	1.27±0.14^{abc}
White cabbage	283.5± 81.7^{abc}	1.35±0.39^{abc}
Leek	296.2±123.4^{ab}	1.41±0.59^{ab}
Radish seed sprout	242.1± 22.8^{abc}	1.15±0.11^{abc}
Experiment 3		
Control	300.7± 85.6^{b}	0.83±0.24^{b}
Propylene glycol	363.9± 16.1^{b}	1.00±0.04^{b}
Broccoli	571.8±105.7^{a}	1.57±0.29^{a}
Turnip	545.9± 22.1^{a}	1.50±0.06^{a}
Green onion	533.8± 22.8^{a}	1.47±0.06^{a}
Radish leaf	509.4± 23.3^{a}	1.40±0.06^{a}
Experiment 4		
Control	248.5± 50.2^{d}	0.80±0.16^{d}
Propylene glycol	313.6± 33.8^{c}	1.00±0.11^{c}
Garlic	490.4± 39.7^{a}	1.57±0.13^{a}
Radish skin	407.5± 75.2^{b}	1.30±0.24^{b}
Kale	393.1± 7.3^{b}	1.26±0.02^{b}
Dried radish leaf	366.1± 44.3^{bc}	1.17±0.14^{bc}

[1)]Solvent extract of fresh vegetables(12g/mouse), garlic(6g/mouse) was administered to 6 to 8-week-old female ICR mice by gavage a daily dose in 5ml/kg weight for 5 days
[2)]Mean±SD
[3)]BHT(1g/kg) dissolved in corn oil(5ml/kg)
[4)]Any two means in the same row with different superscript are significantly different($p<0.05$)

Next, the combination of vegetable extracts was examined for the increase of GST induction(Fig. 1). The combination effect of vegetable extracts on the induction of GST indicates that the combination of broccoli(12g fresh vegetable/mouse) and radish(12g fresh vegetable/mouse) was the most effective, with 1.84-fold induction in GST activities, followed by the combination(1.69-fold) of broccoli and green onion and the combination(1.50-fold) of broccoli and turnip. Induction by the combination of broccoli and radish was slightly higher than that(1.67 to 1.72-fold) derived by the single administration of broccoli or radish at a dose of 24g fresh vegetable based/mouse.

Since sulforaphane was reported to be a major principle responsible for the induction of phase II enzymes such as GST(7), the isothiocyanate present in the extract of vegetables was quantified by GC/MS analysis. The amount of sulforaphane in the extract of vegetables was found to be the highest in broccoli(80.2ppm), followed by turnip(15.4ppm), red cabbage(9.9ppm), radish(8.8ppm) and kale(8ppm). Meanwhile, in the other vegetables examined, the quantity of sulforaphane was negligible. Additionally, the amount of total isothiocyanates except sulforaphane were determined, and were expressed as the percentage of total volatile components(Table 5). Total

Table 4. **Hepatic glutathione S-transferase activity in ICR mice administered with different doses of several vegetable extracts**

Vegetables[1)] \ Dose	12g/mouse	24g/mouse
Radish	1.55±0.21$^{c,2)}$	1.72±0.14^{b}
Broccoli	1.57±0.29$^{ab,3)}$	1.67±0.10^{ab}
Green onion	1.47±0.06^{b}	1.74±0.06^{a}

[1)]Solvent extract of fresh vegetables(12g/mouse or 24g/mouse) was administered to 6 to 8-week-old female ICR mice by gavage a daily dose in(5ml/kg weight) for 5 days
[2)]Mean±SD
[3)]Any two means in the same row and column with different superscript are significantly different($p<0.05$)

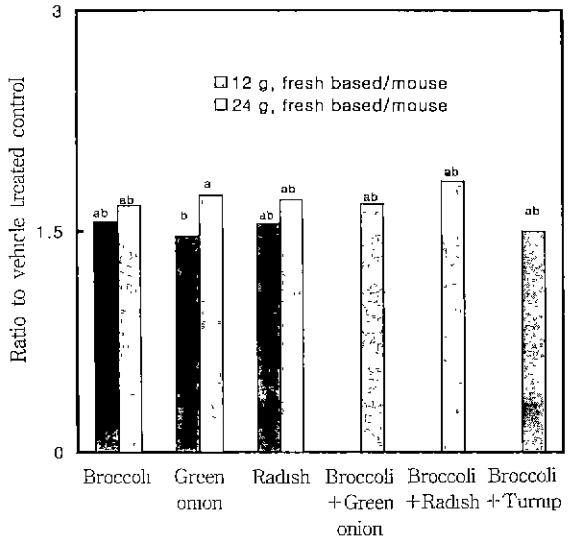

Fig. 1. Combination effect of vegetable extract on the GST activity.
Solvent extract of combination of broccoli(12g/mouse) and green onion(12g/mouse), or broccoli(12g/mouse) and radish(12g/mouse), or broccoli(12g/mouse), and turnip(12g/mouse) was administered to 6 to 8-week-old female ICR mice by gavage at a daily dose(5ml/kg weight) for 5 days.

Table 5. Amount of sulfur and nitrogen containing compounds in solvent extract of vegetables as measured by GC/MS

	Isothiocyanates	Sulfides	Indoles	Nitriles
Broccoli	23.1[1)]	0.6	3.2	–[2)]
Turnip	13.7	2.4	2.9	31.4
Chinese Radish	69.9	0.1	–	–
Red Cabbage	16.8	3.0	9.0	8.8
White Cabbage	6.4	0.1	3.2	0.4
Kale	1.4	–	8.7	0.3
Radish seed sprout	14.7	1.9	3.2	0.3
Dolsan mustard leaf	4.8	–	0.6	1.2
Green Onion	–	7.8	–	0.35
Wild Green onion	–	13.8	–	–
Garlic	–	14.0	–	–
Leek	0.6	4.3	–	–

[1)]Area%
[2)]Not detected

amount of isothiocyanates in the extract of vegetables was determined to be the highest in radish(69.9%), followed by broccoli(23.1%), red cabbage(16.8%), radish seed sprout(14.7%), turnip(13.7%), cauliflower(13.0%), white cabbage(6.4%). Dolsan mustard leaf(4.7%) and mustard leaf(2.7%). Separately, total amount of sulfides in the extract of vegetables was determined, since the sulfide was also reported to induce GST activity. The percentage of sulfides in vegetables was the highest in garlic(14.0%). wild green onion(13.8%), green onion(7.8%), leek(4.3%), red cabbage(3.0%), turnip(2.4%), radish seed sprout(1.87%), broccoli(0.6%) and white cabbage(0.1%). Meanwhile, the percentage of indoles in the extract of vegetables was the highest in red cabbage(9.0%), kale(8.7%), radish seed sprout(3.2%), broccoli(3.2%), white cabbage(3.2%) and turnip(2.9%).

DISCUSSION

Our present results indicate that hepatic GST activity was significantly induced by various vegetable extracts, consistent with previous reports(7,11,12,14,15,20); broccoli was the most effective inducer of GST activity, and a remarkable enhancement was observed with garlic, radish and turnip extracts. Noteworthy, Korean radish extract showed a significant induction(1.55-fold) of GST activity. None of vegetable treatments did have a significant effect on body weight, although there was a suggestion of a significant change of liver weight after the treatment with garlic extract for garlic group. Further study indicates that vegetable extracts containing higher content of sulforaphane are more effective in the induction of GST activity, which is known to be related to the chemoprevention. Thus, there seems to be a positive relationship between GST- induction and the content of sulforaphane in vegetable extracts.

In the previous report(7), synthetic(*R,S*)-sulforaphane (4-methylsufinylbutyl isothiocyanate), erysolin(4-methylsufonylbutyl isothiocyanate) or erucin(4-methylthiobutyl isothiocyanate), administered to female CD-1 mice by garvage, had ben observed to induce QR and GST activity in the cytosols of several organs; sulforaphane and erucin(at daily doses of 15μmole for 5 days) raised both enzyme activities 1.8 to 2.5-folds in liver. This might be supported by our result that approximately 1.8-fold increase of GST activity was observed in cytosol of liver of mice administered with vegetable extracts rich in sulforaphane. No remarkable difference of GST induction between two doses, 12g fresh vegetable/mouse and 24g fresh vegetable/mouse, implies that the GST-induction may be close to a maximal level at the doses used. However, our results do not rule out the possibility of the GST induction by other constituents, since garlic extract, lacking in sulforaphane, also expressed a remarkable GST induction. Therefore, the GST induction could be ascribed to the presence of other constituents in addition to sulforaphane.

Earlier, extracts of onion family vegetables such as garlic or onion were reported to induce GST activity, and the induction of GST activity was related to the presence of sulfides or disulfides(21,22). It is noteworthy that garlic extract showed the highest amount of sulfides. Garlic oil, which was showed an anticarcinogenic activity (22), was observed to contain bioactive organosulfur compounds such as diallylsulfide, allylmethyldisulfide or diallyltrisulfide. Specially, diallylsulfide was a profound inducer of enzymes such as CYP2B, CYP3A1 and epoxide hydrolase, although the induction of GST activity differed according to authors(20,21).

There are two types of anticarcinogenic enzyme inducers: (a) bifunctional inducers that elevate both phase II enzymes(e.g., glutathione S-transferase, UDP-glucuronosyltransferases and quinone reductase) and phase I enzymes(e.g., cytochrome P_{450}); and (b) monofunctional inducers that elevate primarily phase II enzymes without significantly affecting cytochrome P_{450}(23). Isothiocyanates may be related to the capacity to induce phase II enzymes, a characteristic of monofunctional inducer. Es-

pecially, sulforaphane, which occurs naturally in a widely consumed vegetables, elevates phase II detoxification enzymes without significantly changing the elevation of cytochrome P_{450}(7). Meanwhile, disulfides such as diallylsulfide are known to induce both phase I enzymes and phase II enzymes, a characteristic of bifunctional inducer. In this relation, the combination of phase I and phase II inducer vegetables was expected to increase the GST induction beyond that by phase I inducer vegetables or phase II inducer vegetables only. However, the combination of broccoli and green onion was less effective than that of broccoli and radish. Thus, there seems to be no synergism between phase II inducer vegetables and phase I inducer vegetables in inducing GST activity. Despite this, the combination of phase I inducer and phase II inducer vegetables could have an advantage in the detoxification of xenobiotics, since phase I and Phase II enzymes are independently involved in the metabolism of xenobiotic compounds to produce excretory metabolites (3,23). Phase I enzymes introduce polar groups into xenobiotic compounds, and the presence of a polar group on a xenobiotic compound provides a means by which a subsequent conjugation by phase II enzyme reaction can occur, leading to excretion. Therefore, a proper combination of phase I enzyme inducer and phase II enzyme inducer would be a choice for the maximal detoxification of xenobiotic compounds including chemical carcinogens. In this respect, the effect of combination of monofunctional inducer vegetables and bifunctional inducer vegetables on detoxification of chemical carcinogenic compounds remains to be investigated in the future.

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