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Induction of GST and NQO1 in Cultured Bladder Cells and in the Urinary Bladders of Rats by an Extract of Broccoli (*Brassica oleracea italica*) Sprouts

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Deficiency of glutathione S-transferase (GST) or NAD(P)H:quinone oxidoreductase 1 (NQO1) in humans is associated with increased risk of urothelial bladder cancer. Broccoli sprouts are a rich source of several isothiocyanates (ITCs), particularly sulforaphane (SF) which has shown promising chemopreventive activities. We report herein that a broccoli sprout ITC extract significantly induced both GST and NQO1 in cultured bladder cells in vitro and in rat bladder tissues in vivo. The inducer activity of the extract was comparable to that of pure SF on the basis of total ITC concentrations. The bladder was one of the most responsive organs to induction of the enzymes by the extract. Induction of the enzymes by the extract was largely mediated by Nrf2, a transcriptional factor that plays a critical role in the induction of many detoxification enzymes. Moreover, induction of GST and NQO1 in the rat bladder in vivo by the extract was associated with high levels of urinary ITC metabolites, but no toxic effects on the bladder mucosa were detected. In conclusion, broccoli sprout ITC extract is a potent inducer of GST and NQO1 in the bladder and is a promising agent for prevention of bladder cancer.

KEYWORDS: Phase 2 enzyme; bladder cancer chemoprevention; isothiocyanate; sulforaphane; cruciferous vegetable

INTRODUCTION

Both glutathione S-transferase (GST) and NAD(P)H:quinone oxidoreductase 1 (NQO1) are well-known cytoprotective enzymes. GST is composed of multiple cytosolic and membrane-bound isoforms and primarily functions as a detoxification enzyme by catalyzing the conjugation of glutathione with a wide range of carcinogens and other toxic chemicals (*1*). NQO1, a cytosolic flavoprotein, is best known for protecting cells against the toxicities of quinones and their metabolic precursors by catalyzing obligatory two-electron reduction of these compounds, as well as acting as a coenzyme Q (ubiquinone) reductase and facilitating the conversion of α-tocopherolquinone to α-tocopherolhydroquinone, contributing to the maintenance of these important endogenous antioxidants (*2*, *3*). More recently, it has been shown that NQO1 is involved in stabilizing tumor suppressor p53 (*3*), suggesting that this protein may play a role in many aspects of cytoprotection. The importance of these enzymes in cancer prevention has been demonstrated in several animal models. Knockout of either GSTP1 (a GST isoform) or NQO1 in mice led to a significant increase in both carcinogen-induced and spontaneous tumorigenesis (*4*−*7*). Epidemiological studies in humans have shown an increased risk of urothelial and bladder cancers in individuals who carry a null genotype or a genotype that causes a significant decrease in enzyme activity of a GST isoform or NOQ1 (*8*−*13*).

Both GST and NQO1 are inducible enzymes. We have previously shown that many naturally occurring isothiocyanates (ITCs), including the ITCs that occur in broccoli sprouts, significantly increase the activities of GST and NQO1 in both cultured bladder cells and rat bladder tissue in vivo (*14*−*16*). Moreover, these studies also showed that the bladder was particularly responsive to ITCs in the induction of these enzymes. It is possible, therefore, that ITCs could be especially effective in protecting against bladder cancer.

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In the present study, we have examined the effect of a broccoli sprout extract, which is rich in sulforaphane (SF, 4-methylsulfinylbutyl isothiocyanate) and which also contains two close analogs, iberin (3-methylsulfinylbutyl isothiocyanate) and erucin (4-methylthiobutyl isothiocyanate) (*17*), on GST and NQO1 in both cultured cells and rat organs. We show that (1) SF-rich broccoli sprout extract is a strong inducer of both GST and NQO1 in cultured bladder cells in vitro and in rat bladder in vivo; (2) the bladder is one of the most responsive tissues for induction of the enzymes by the extracts; (3) how the extract is administered to rats has a significant impact on its enzyme-inducing efficacy in the bladder; (4) the induction of GST and NQO1 by the extract depends primarily on Nrf2, a transcription factor that is critical for stimulation of many cytoprotective genes; and (5) induction of GST and NQO1 in the bladder by SF-rich broccoli sprout extract is associated with high urinary concentrations of ITC metabolites but is not accompanied by any toxic effect on the bladder mucosa.

MATERIALS AND METHODS

Chemicals. SF was purchased from LKT Laboratories (St Paul, MN). Lyophilized SF-rich broccoli sprout ITC extract was prepared from 3-day-old fresh broccoli sprouts and was stored at −70 °C before use. The preparation of the extract and the characterization of its ITC content has been previously described (*18*). Briefly, broccoli sprouts were grown by Sprouters Northwest (Kent, WA) using seeds from an open-pollinated population based upon *Brassica oleracea italica* cultivars DeCicco and Calabrese which were purchased from Caudill Seed Co., Inc. (Louisville, KY). Fresh sprouts were added to boiling water at a ratio of 100 kg sprouts per 300 liters of water, in a steam-jacketed kettle with stirring. The sprouts were boiled for 30 min and then were filtered through a stainless-steel mesh screen. The filtrate contains the glucosinolate precursors of ITCs. Conversion of these glucosinolates to ITCs was effected by adding daikon sprouts-derived myrosinase to the filtrates and incubating at 32−38 °C for 3.5 h. The mixture was transferred to trays and was freeze-dried to yield 2.8 kg powder (from 100 kg broccoli sprouts). Each gram of extract contained 140 μmol ITC, of which 70%, 25%, and 5% were SF, iberin, and erucin, respectively, as determined by high-performance liquid chromatography.

Cell Culture and Measurement of Cellular GST and NQO1 Activities. The source and culture condition of rat bladder carcinoma NBT-II cells have been previously described (*19*). Wild-type ($Nrf2^{+/+}$) and Nrf2-deficient ($Nrf2^{-/-}$) mouse embryonic fibroblasts (MEF) were gifts of Dr. Masayuki Yamamoto, University of Tsukuba, Japan (*20*). These cells were maintained in Iscoves Modified Dulbecco's Medium (Mediatech, Herndon, VA), containing 10% heat-inactivated fetal bovine serum (FBS, v/v), 100 units/mL penicillin G, and 0.01% streptomycin. For heat inactivation, FBS (Biosource International, Camarillo, CA) was incubated at 55 °C for 30 min. All cells were grown in a humidified incubator at 37 °C with 5% CO_2.

These cells were used to evaluate the effect of broccoli sprout extract and SF on the activities of cellular GST and NQO1. Cells were seeded in 10-cm dishes (1×10^6 cells/dish with 10 mL medium) for 48 h and then were treated with the test agent in fresh medium for 24 h. At the end of the treatment, cells were harvested by trypsinization and centrifugation and were lysed by sonication in 0.08% digitonin solution containing 2 mM EDTA. The lysates were centrifuged at 9500*g* at 4 °C in a microfuge, and the supernatant portions were used for enzyme analysis, using 1-chloro-2,4-dinitrobenzene as a GST substrate and menadione as an NQO1 substrate, as previously described (*21*).

Animals. Female Sprague−Dawley rats (11−12 weeks of age), bred at the Ruakura Agricultural Research Center (Hamilton, New Zealand), were used to study the effect of broccoli sprout extract on tissue GST and NQO1 activities and on the urinary excretion levels of ITC metabolites. In the study on enzyme induction, rats were housed in solid bottom cages, containing bedding of softwood shavings, and were allowed free access to AIN76A purified diet (Bio-Serv, Frenchtown, NJ) and water. For the urinary excretion study, the animals were individually housed in metabolism cages for urine collection, and the animals had free access to pelletized Laboratory Chow (Sharpes Animal Feeds, Carterton, NZ) and water. The temperature of the animal room was maintained at 21−23 °C with a 12-h light/dark cycle. Animal body weights were monitored every 2−3 days. All experimental protocols were approved by the Ruakura Animal Ethics Committee.

Administration of Broccoli Sprout Extract to Rats, Determination of Tissue NQO1 and GST Activities, and Measurement of Urinary Levels of ITC Metabolites. The effect of broccoli sprout extract on tissue activities of GST and NQO1 in rats was assessed using two dosing protocols: oral intubation and dietary supplementation. In the former experiment, the extract was suspended in water at the required concentrations and was administered by intubation to groups of six rats in approximately 0.5 mL volume once daily for 14 days. The rats were weighed immediately before each dosing, and the volume of suspension to be administered to give the required dose was calculated. The animals were killed 24 h after the last dose by CO_2 inhalation. In the second experiment, the extract was mixed with the diet, which was prepared fresh every 2−3 days, and was fed to groups of six rats for 14 days. On the 15th day, the rats were killed by CO_2 inhalation. The bladder, colon, duodenum, forestomach, kidneys, liver, and lungs of each animal were removed and stored at −80 °C before analysis. One-half of each bladder was stored frozen, while the other half was fixed in 10% buffered formalin for histological examination.

For measurement of enzyme activities, all organ samples were weighed and homogenized in ice-cold 0.2% Triton X-100, using a Polytron tissue homogenizer. The homogenates were centrifuged at 12 000*g* for 20 s, and the supernatant was assayed for GST and NQO1 activities by the method of Habig et al. (*22*) and Ernster (*23*), respectively. Enzyme activities were calculated as international units per gram of tissue.

To measure urinary levels of ITC metabolites, groups of five rats were given a single dose of broccoli sprout extract by oral intubation to provide ITC doses of 10, 25, 50, and 250 μmol/kg body weight, and two consecutive 24-h urines were subsequently collected from each rat. Urine samples were stored at −70 °C before analysis. Total urinary levels of ITC metabolites were measured with the cyclocondensation assay (reaction with 1,2-benzenedithiole) (*24*), using a previously described protocol (*25*). This assay measures the total amount of ITCs plus their metabolites (dithiocarbamates, DTCs) formed in the mercapturic acid pathway, and it does not provide information on the amount of individual compounds in the urine samples.

Histological Examination of Bladders of Rats Dosed with Broccoli Sprout Extract. Rat bladders were fixed in routine histology fixative (buffered formalin), were placed in an automated tissue processor, and were processed on a biopsy (5 h) schedule because of their small size. After the tissues were embedded in paraffin, they were sectioned at 5 μm and were placed on charged slides. The tissues were stained with hematoxylin and eosin (H&E) for microscopic examination.

Statistical Analysis. Statistical significance was tested by analysis of variance, followed by the Student−Newman−Keuls multiple comparisons test using Instat software (GraphPad, San Diego, CA).

RESULTS

Induction Levels of GST and NQO1 in the Bladder Vary Significantly with the Method of Administration of Broccoli Sprout Extract to Rats. In the first experiment, broccoli sprout extract was suspended in water and was administered to groups of rats by oral intubation in amounts calculated to provide 40 and 160 μmol ITC/kg body weight once daily for 14 days. The extract caused a significant and dose-dependent induction of both GST and NQO1 in the bladder, although the induction levels of GST were somewhat lower than those of NQO1. GST and NQO1 activities in the bladder were elevated by 43% and 141%, respectively, at 40 μmol ITC/kg body weight and by 105% and 337% at 160 μmol ITC/kg (**Figure 1A**). In the second experiment, the sprout extract was mixed in the diet and was fed to rats at 0.53, 1.06, and 2.13 μmol ITC/g diet, respectively, for 14 days. Another group of rats received control diet alone.

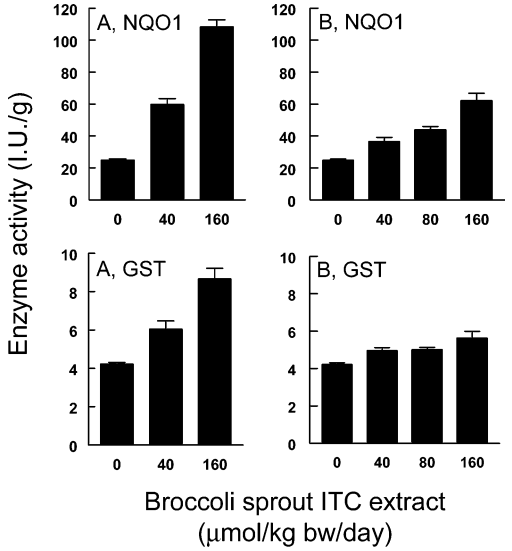

Figure 1. Induction of GST and NQO1 in rat bladder by broccoli sprout ITC extract. Rats received broccoli sprout extract by oral intubation (**A**) or by supplementation in the diet (**B**) as described in Materials and Methods. Each value is the mean ± SEM ($n = 6$). Each value from extract-treated rats is significantly higher than the control ($p < 0.05$).

The rats in this study weighed 238 ± 12 g and consumed 16.2 ± 1.3 g of diet daily. Hence, the average daily ITC consumption levels were calculated to be 0, 40, 80, and 160 μmol ITC/kg body weight, respectively. The broccoli sprout extract fed to the rats in the diet also caused a significant and dose-dependent induction of both GST and NQO1 in the bladder, and NQO1 was again more responsive than GST (**Figure 1B**). For example, the activities of GST and NQO1 in the bladder were elevated 18% and 48% at 40 μmol ITC/kg and 33% and 151% at 160 μmol ITC/kg. However, these levels of enzyme induction were lower than those obtained when the same amount of extract was given by oral intubation, as shown above. Interestingly, such an effect of dosing method appears to be highly organ specific, since the forestomach was the only other organ that behaved in this fashion among the seven rat organs examined (**Figure 2**).

Bladder Is among the Most Responsive Organs in the Induction of GST and NQO1 by Broccoli Sprout Extract. Our previous studies have shown that many ITCs are more efficacious inducers of GST and NQO1 in the bladder than in other organs (*14*−*16*). In the present study, the activities of GST and NQO1 in several organs from rats dosed with the extract, including colon, duodenum, forestomach, kidney, liver, and lungs, were measured and compared with those in the bladder. Shown in **Figure 2** are the results obtained from rats dosed with the extract at 40 and 160 μmol ITC/kg. The duodenum appears to be the most sensitive responder; its enzyme induction levels were the highest among the organs examined and did not appear to vary with how the extract was dosed. While the enzyme induction levels in the bladder and forestomach were lower than in the duodenum when the extract was provided in the diet, they were similar to those in the duodenum when the extract was dosed by oral intubation. In contrast, the induction levels of both GST and NQO1 in colon, kidney, liver, and lungs were relatively small, regardless of the dosing method.

Induction of GST and NQO1 in Bladder Cells and Tissues by Broccoli Sprout Extract May Be Caused Exclusively by ITCs. We recently showed that the ITCs in broccoli sprout extract accounted for the antiproliferative activity of this material in cultured human bladder cancer cells and that such activity

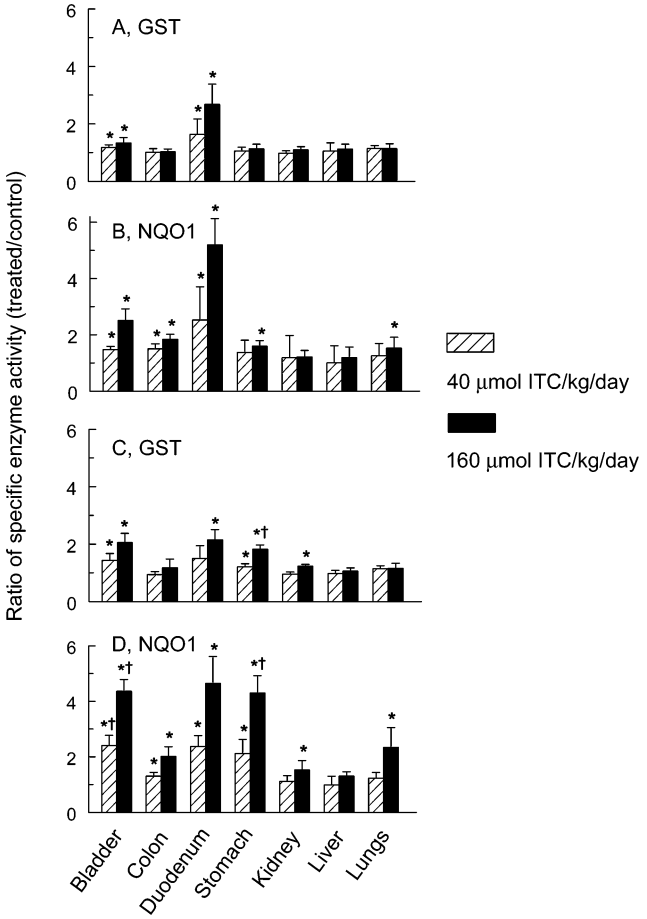

Figure 2. Comparison of induction levels of GST and NQO1 by broccoli sprout ITC extract among rat organs. Broccoli sprout extract was administered to the rats either by dietary supplementation (**A** and **B**) or by oral intubation (**C** and **D**) as described in Materials and Methods. Each value is the mean ± SEM ($n = 6$). Each value marked with an asterisk is significantly different from the control ($p < 0.05$). Values marked with a dagger in **C** and **D** are significantly greater than the corresponding values in **A** and **B** ($p < 0.05$).

was similar to that of SF when related to ITC concentrations (*18*). In the present study, we compared the inducer activity of broccoli sprout extract with that of SF in rat bladder carcinoma NBT-II cells. Cells were treated with either the extract or with SF for 24 h and then were analyzed for induction of both GST and NQO1. The extract, at concentrations that provided 4−8 μM ITC, elevated the activities of GST and NQO1 by 62−78% and 106−145%, respectively, whereas SF at the same concentrations increased the activities of GST and NQO1 by 77−108% and 127−173%, respectively (**Figure 3**). Thus, the inductive activity of the extract was only slightly weaker than SF on the basis of the ITC concentrations. Similar results were obtained in cultured human bladder cells (results not shown). In light of our previous study (*26*), which showed that both iberin and erucin, the minor ITCs in broccoli sprout extract, were weaker inducers of NQO1 in cultured murine hepatoma Hepa 1c1c7 cells than SF, and because of our previous demonstration that the NQO1-inducing activity of broccoli sprout extract in Hepa 1c1c7 cells depended entirely on the presence of ITCs (*17*), the present results suggest that the enzyme-inducing activity of broccoli sprout extract in bladder cells is derived exclusively from the ITCs.

Induction of GST and NQO1 by Broccoli Sprout Extract Depends Largely on Nrf2. It is well recognized that many carcinogen-detoxifying enzymes are transcriptionally activated

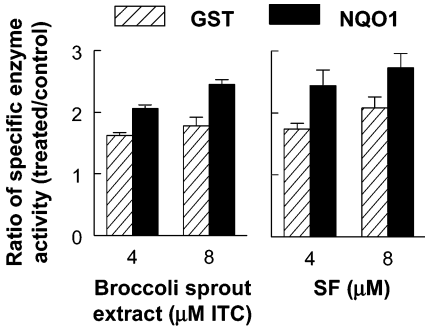

Figure 3. Induction of GST and NQO1 in rat bladder NBT-II cells by broccoli sprout ITC extract and SF. Cells were treated with either broccoli sprout extract or SF at the indicated ITC concentrations for 24 h and then were harvested for determination of specific GST and NQO1 activities as described in Materials and Methods. Each value is the mean ± SEM ($n = 3$). Each value with treatment of the sprout extract and SF is significantly different from the control ($p < 0.05$).

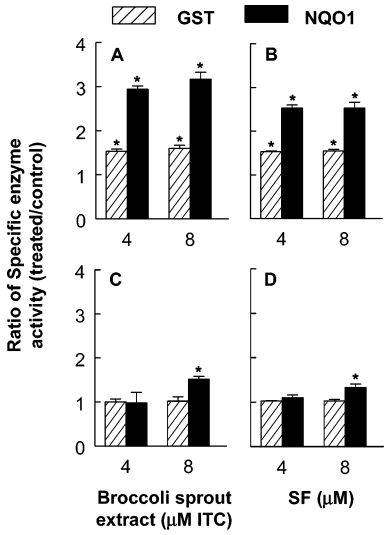

Figure 4. Induction of GST and NQO1 by broccoli sprout ITC extract and SF in wild-type and Nrf2 knockout MEF. Cells were treated with either broccoli sprout extract or SF at the indicated ITC concentrations for 24 h and then were harvested for determination of specific GST and NQO1 activities as described in Materials and Methods. **A** and **B**, wild-type MEF; **C** and **D**, Nrf2 knockout MEF. Each value is the mean ± SEM ($n = 3$). Values marked with an asterisk are each significantly higher than the control ($p < 0.05$).

through an Nrf2-dependent signaling pathway (*27*). Nrf2 is a transcription factor which is normally suppressed in the cytoplasm by its repressor Keap1, but chemical modification of the latter by inducers leads to nuclear accumulation of Nrf2 (*28*, *29*), where it stimulates gene transcription by binding to ARE (antioxidant response element), a DNA regulatory element that exists in the upstream region of genes, including GST and NQO1. ITCs, as well as other inducers, have been shown to activate this signaling pathway (*30*). SF potently induced both GST and NQO1 in wild-type mouse embryonic fibroblasts (MEF), but Nrf2 knockout totally abolished GST induction and dramatically attenuated NQO1 induction (**Figure 4**). The broccoli sprout extract behaved similarly, causing strong induction of both GST and NQO1 in wild-type MEF but small (NQO1) or no (GST) induction in Nrf2 knockout MEF. Similar results were obtained with higher doses of the extract and SF (results not shown).

Table 1. Urinary Excretion of Orally Administered Broccoli Sprout ITCs in Rats

			urinary ITC plus DTC	
ITC dose (μmol/kg b.w.)	urine collection period post ITC dosing	total urine volume (mL)	concn (μM)	% recovery
10	0–24 h	13.6 ± 1.4[a]	113.3 ± 19.4[a]	70.4
	25–48 h	10.9 ± 0.4	2.5 ± 0.5	1.4
25	0–24 h	15.9 ± 1.8	243.9 ± 22.1	71.8
	25–48 h	10.9 ± 1.4	5.6 ± 1.4	1.4
50	0–24 h	12.4 ± 2.1	702.2 ± 88.6	78.8
	25–48 h	11.9 ± 1.0	10.4 ± 1.7	1.2
250	0–24 h	13.9 ± 1.9	3405.3 ± 470.3	78.2
	25–48 h	13.7 ± 1.8	41.9 ± 5.9	1.1

[a] Mean ± SEM ($n = 5$). Each Sprague–Dawley rat received a single oral dose of broccoli sprout extract.

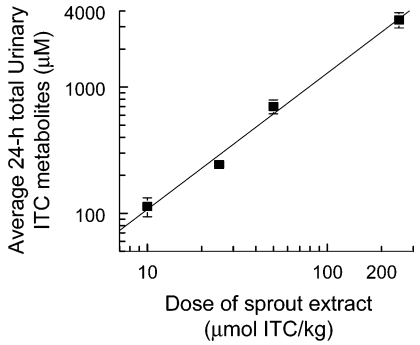

Figure 5. Urinary excretion levels of ITC metabolites in rats following administration of broccoli sprout ITC extract. The data are drawn from a part of the results shown in **Table 1**. Each rat was given a single oral dose of broccoli sprout extract, and urine was collected for 24 h postdosing. Each value is the mean ± SEM ($n = 5$). Urinary elimination of ITC metabolites was linearly proportional to the ITC dose ($r^2 = 0.992$).

The Amount of Broccoli Sprout Extract Required To Induce GST and NQO1 in Vivo Generates High Urinary Levels of ITC Metabolites. Groups of five rats were given a single dose of the extract by oral intubation at amounts sufficient to provide doses of 10, 25, 50, and 250 μmol ITC/kg. Urine was subsequently collected from each rat for two consecutive 24-h periods. While urine from control rats contained no ITC metabolites (results not shown), 70.4−78.8% of the ITC doses provided by the broccoli sprout extract were eliminated as cyclocondensation assay-detected substances (ITC plus DTC) in the first 24-h urine and 1.1−1.4% of the doses in the second 24-h urine (**Table 1**), indicating extremely high bioavailability and predominant and rapid urinary disposition of broccoli sprout ITCs. It is also clear that the urinary ITC elimination process is rather robust, as the urinary ITC elimination rate was linear up to 250 μmol ITC/kg (**Figure 5**). Moreover, the results show that the bladders were exposed to very high concentrations of ITC plus DTC in the urine when the animals were dosed with the extract at the levels that are needed for significant induction of GST and NQO1 in this organ. For example, on the basis of the urinary elimination pattern shown in **Figure 5**, we calculated that the average urinary concentrations of ITC plus DTC in the first 24 h following oral intubation of single dose of extract at 40 and 160 μmol ITC/kg, which significantly induced GST and NQO1 in the bladder, were 480 μM and 2.3 mM. Importantly, none of the extract doses tested in the present study were associated with any sign of toxicity. All rats appeared in good health, and body weight gains over the course of the experimental period were not significantly different among the

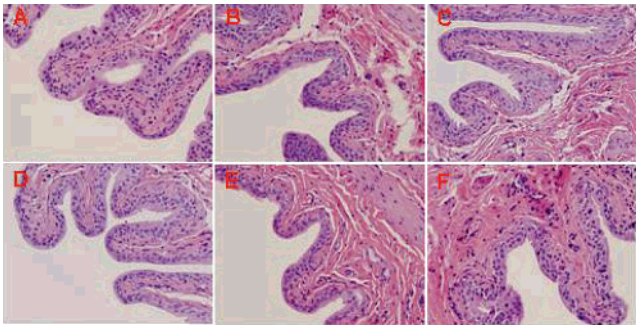

Figure 6. H&E stained bladder tissues of rats after administration of broccoli sprout ITC extract. Rats were given broccoli sprout ITC extract either in the diet or by oral intubation daily for 14 days. On the 15th day, the rats were killed, and the bladders were removed and processed for microscopic examination of bladder tissue. **A**, control; **B**, 40 μmol ITC/kg b.w./day, extract in the diet; **C**, 80 μmol ITC/kg b.w./day, extract in the diet; **D**, 160 μmol ITC/kg b.w./day, extract in the diet; **E**, 40 μmol ITC/kg/day, oral intubation; **F**, 160 μmol ITC/kg/day, oral intubation. The images are representative of bladders of other rats in the same groups (six rats/group).

treatment groups and the control group (results not shown). No gross abnormalities were detected at necropsy. When the rat bladder tissues were examined microscopically after H&E staining, no pathological changes were visible (**Figure 6**).

DISCUSSION

Several lines of evidence show that deficiency of GST or NQO1 increases the risk of bladder cancer in humans. Our present study shows that broccoli sprout extract causes significant induction of both enzymes in cultured bladder cells and in the bladder of rats in vivo. Moreover, the inducer efficacy of this extract in cultured bladder cells in vitro (**Figure 3**) and in the rat bladder in vivo was very similar to that of SF (*15*). SF is the principal ITC in the extract (*18*) and is a well-known cancer chemopreventive agent (*31*). These results suggest that broccoli sprout extract is not only potentially useful for bladder cancer prevention but also is an excellent substitute for SF. In this context, it is important to note that fresh broccoli sprouts may not be as good a delivery vehicle of SF as broccoli sprout extract. SF and other ITCs in broccoli sprouts are generated through myrosinase-catalyzed hydrolysis of their glucosinolate precursors (β-thioglucoside *N*-hydroxysulfates). However, myrosinase-induced ITC formation in broccoli sprouts is in competition with the activity of epithiospecifier protein (ESP), which converts the intermediate products of glucosinolate hydrolysis (thiohydroximate-*O*-sulfonates) to nitriles and other products, which are not known to possess chemopreventive activity (*32*, *33*). In fresh broccoli sprouts, only a small fraction (~17%) of the glucosinolates were converted to ITCs (our unpublished data), as both myrosinase and ESP were fully active, although mild heating increases ITC yield because ESP is more heat-sensitive than myrosinase (*32*). In the preparation of broccoli sprout extract, however, endogenous enzymes and ESP are inactivated by heating, and a high yield of ITCs is ensured by subsequently adding a preparation of myrosinase uncontaminated with ESP.

Our studies also show that Nrf2 plays a critical role in GST and NQO1 induction by the broccoli sprout extract. Knockout of Nrf2 in MEF completely abolished GST induction and markedly attenuated NQO1 induction (**Figure 4**). The dependence of the extract on Nrf2 in GST and NQO1 induction was similar to that of SF, which was previously shown to depend on Nrf2 for induction of many detoxification enzymes (*34*). However, there was a small but significant induction of NQO1 after treatment of Nrf2-deficient MEF with either the extract or SF, suggesting that an Nrf2-independent mechanism may also mediate to a minor extent the induction of this enzyme. Nrf2-related factors such as Nrf1 and Nrf3 have been reported to mimic the function of Nrf2 (*35*, *36*). However, it seems unlikely that these factors play a role in NQO1 induction observed in the present study, because Nrf2 knockout completely abolished GST induction. Rather, we speculate that it may involve activator protein 1 (AP-1). Although we are unable to find any literature reports of NQO1 regulation by AP-1 in murine cells and tissues, a functional AP-1 binding site is known to exist in the 5′-flanking region of the human NQO1 gene (*37*, *38*). We and others have previously shown that ITCs, including SF, stimulate trans-activation of AP-1 in various cultured cell lines (*19*, *39*, *40*).

Bladder, duodenum and forestomach were the most responsive organs in the induction of GST and NQO1 in vivo by the broccoli sprout extract. However, the efficacy of this extract in the bladder was markedly influenced by dosing method, as oral intubation of the extract was significantly more effective than its supplementation in the diet (**Figure 1**). Interestingly, similar effects were also seen in the forestomach, but not in other organs examined (**Figure 2**). Both bladder and forestomach may be viewed as organs that temporarily store ITCs or their metabolites, because ingested ITCs may be kept in the stomach for some time before being passed to the small intestine, whereas urinary metabolites of ITCs (mainly *N*-acetylcysteine (NAC) conjugates), which can undergo time-dependent dissociation to ITCs, are stored in the bladder until urine discharge. Thus, it is conceivable that bolus ITC administration maximizes the exposure of these organs to ITCs or their metabolites. Also, the duodenum remained one of the most responsive organs, perhaps the most responsive among the organs examined, in the induction of GST and NQO1, regardless of how the broccoli sprout extract was administered, suggesting that broccoli sprout extracts may be an excellent cytoprotective substance in this tissue. The reason for the sensitivity toward induction of GST and NQO1 by the broccoli sprout extract in the duodenum is presently unknown.

ITCs are known to be metabolized in vivo mainly through the mercapturic acid pathway and to be excreted in the urine as NAC conjugates. Using the cyclocondensation assay, which measures free ITCs, NAC conjugates, and the intermediate metabolites in the mercapturic acid pathway (collectively termed DTC), we showed extensive (70.4−78.8%) elimination of the ITC doses provided by the broccoli sprout extract within 24 h. Little more ITCs or their derivatives were excreted in the subsequent 24 h. These results are consistent with those of Kassahun et al. (*41*), who showed that 72% of a single oral dose of SF was eliminated within 24 h as NAC conjugates in the urine of rats. Similar results were also seen in humans dosed with broccoli or broccoli sprouts (*42*, *43*). Interestingly, the urinary concentrations of ITC metabolites associated with significant induction of GST and NQO1 in the bladder appear to be dramatically higher than those required for induction in cultured cells. While broccoli sprout extract providing ITC concentrations of 4 and 8 μM significantly induced GST and NQO in cultured NBT-II cells and MEF, the urinary concentrations of total DTC, the majority of which are presumably NAC conjugates, in rats that were fed with amounts of the ITC extract that caused significant induction of GST and NQO1 in the bladder were 2−3 orders of magnitude higher. This suggests a

very limited dissociation of NAC-ITCs to ITCs in the urine and/or very limited uptake of ITCs/NAC-ITCs from urine by bladder mucosa. Our previous study suggests that intact NAC-ITCs are unable to enter cells (*44*). This suggestion is in accord with our finding that urinary total ITC metabolites up to 3.4 mM did not cause any detectable injury to the bladder tissue.

In summary, broccoli sprout extract is a strong inducer of GST and NQO1 in cultured bladder cells in vitro and rat bladder in vivo. The bladder is among the most responsive organs with respect to the induction of these enzymes by the extract. GST induction by the ITC extract appears to be entirely Nrf2-dependent, whereas NQO1 induction may involve both Nrf2-dependent and -independent mechanisms. While urinary concentrations of ITC metabolites in the rats dosed with the ITC extract in association with significant GST and NQO1 induction in the bladder are extremely high, no toxic effects on the bladder tissue were observed.

ABBREVIATIONS USED

DTC, dithiocarbamate; GST, glutathione S-transferase; ITC, isothiocyanate; MEF, mouse embryonic fibroblast; NAC, *N*-acetylcysteine; NQO1, NAD(P)H:quinone oxidoreductase 1; SF, sulforaphane.

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