British Journal of Nutrition (2013), **109**, 25–32
doi:10.1017/S0007114512000657

Organ-specific exposure and response to sulforaphane, a key chemopreventive ingredient in broccoli: implications for cancer prevention

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(Submitted 15 December 2011 – Final revision received 30 January 2012 – Accepted 31 January 2012 – First published online 2 April 2012)

Abstract

Naturally occurring sulforaphane (SF) has been extensively studied for cancer prevention. However, little is known as to which organs may be most affected by this agent, which impedes its further development. In the present study, SF was administered to rats orally either in a single dose or once daily for 7 d. Tissue distribution of SF was measured by a HPLC-based method. Glutathione *S*-transferase (GST) and NAD(P)H:quinone oxidoreductase 1 (NQO1), two well-known cytoprotective phase 2 enzymes, were measured using biochemical assays to assess tissue response to SF. SF was delivered to different organs in vastly different concentrations. Tissue uptake of SF was the greatest in the stomach, declining rapidly in the descending gastro-intestinal tract. SF was rapidly eliminated through urinary excretion, and urinary concentrations of SF equivalents were 2–4 orders of magnitude higher than those of plasma. Indeed, tissue uptake level of SF in the bladder was second only to that in the stomach. Tissue levels of SF in the colon, prostate and several other organs were very low, compared to those in the bladder and stomach. Moreover, induction levels of GST and NQO1 varied by 3- to 6-fold among the organs of SF-treated rats, though not strictly correlated with tissue exposure to SF. Thus, there is profound organ specificity in tissue exposure and response to dietary SF, suggesting that the potential chemopreventive benefit of dietary SF may differ significantly among organs. These findings may provide a basis for prioritising organs for further chemopreventive study of SF.

Key words: Chemoprevention: Nutrition: Phase 2 enzymes: Sulforaphane

Sulforaphane (1-isothiocyanato-4-methylsulfinylbutane, SF) was shown in the early 1990s as a key cancer chemopreventive ingredient in broccoli[1,2] and later found to be particularly abundant in broccoli sprouts[3]. Although it was initially shown to be a potent inducer of phase 2 cytoprotective enzymes via Nrf2 activation, subsequent studies have revealed that SF imparts pleotropic anticancer effects, modulating genes and processes involved in many cancer-related events, including susceptibility to carcinogens, cell cycle, cell death, angiogenesis, invasion, metastasis and cancer stem cell growth[4–6]. Numerous studies have been carried out to evaluate the anticancer potential of SF. In animal studies, SF or SF-containing broccoli sprout extract has been shown to inhibit cancer development and/or progression in a wide variety of organs, including bladder[7], breast[2,6], colon[8,9], lung[10], pancreas[11], prostate[12], skin[13,14] and stomach[15]. Other studies employing subcutaneous xenograft cancer models have showed that SF could also inhibit the growth of oesophageal cancer[16], osteosarcoma[17], pancreatic cancer[5] and prostate cancer[18,19]. These and other findings have generated a widespread interest in SF and have prompted speculation that the anticancer activity of SF may not be organ-specific. Indeed, human chemopreventive studies of SF using broccoli sprouts or broccoli sprout extracts have been attempted in the breast[20], liver[21], skin[22], stomach[23] and upper airway[24]. However, many lines of evidence on the metabolic disposition of SF *in vivo* as described next suggest that dietary SF may reach different organs in different concentrations.

SF is primarily metabolised via the mercapturic acid pathway *in vivo*; it first reacts with glutathione to give rise to the corresponding conjugate, which is further metabolised to cysteinylglycine conjugate, cysteine conjugate, and finally *N*-acetylcysteine conjugate, which are rapidly disposed in the urine[4]. In both humans and rats, approximately 70 % of orally administered SF, whether pure or via broccoli sprout extracts, is eliminated via the mercapturic acid pathway within 12–24 h[7,25,26]. The SF metabolites serve as its carriers, as they are unstable and readily dissociate to the parent compound. Conaway *et al.*[27] have shown that the SF metabolites formed via the mercapturic acid pathway are unstable and under physiological conditions dissociate back to SF with a

Abbreviations: GST, glutathione *S*-transferase; NQO1, NAD(P)H:quinone oxidoreductase 1; SF, sulforaphane.

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half-dissociation time ranging from 14 min (the cysteine conjugate of SF) to 173 min (the *N*-acetylcysteine conjugate of SF). In human and rat studies involving broccoli sprout extract, plasma concentrations of SF equivalents were highest approximately 1 h after oral dosing and declined rapidly thereafter, while concentrations of SF equivalents in urine were markedly higher than in the plasma[7,28]. These findings suggest that bladder exposure to orally ingested SF is greater than that of many other organs. Indeed, our previous study showed that tissue induction levels of two well-known cytoprotective phase 2 enzymes, glutathione *S*-transferase (GST) and NAD(P)H:quinone oxidoreductase 1 (NQO1), were significantly higher in the bladder than in many other organs in rats fed with broccoli sprout extracts[29]. The same study showed that the levels of GST and NQO1 induced by broccoli sprout extracts in the stomach and duodenum were also relatively high, suggesting that exposure of the upper gastrointestinal tract to dietary SF may be high, possibly reflecting high concentrations of SF in these tissues after oral administration, before absorption.

In the present study, we examined *in vivo* tissue exposure and response to dietary SF in a large number of organs of rats. GST and NQO1 were measured as biomarkers of tissue response to SF. Both GST and NQO1 are ubiquitously expressed cytoprotective phase 2 enzymes that are readily induced by SF[4]. Concentrations of SF equivalents in the plasma and urine were also measured. Our study results show that tissue exposure and response to dietary SF differ dramatically among organs.

Experimental methods

Chemicals

R,*S*-SF (≥98 % purity) and 1,2-benzenedithiol (97 % purity) were purchased from LKT Laboratories and Alfa Aesar, respectively. Reagents for enzyme assays were all ≥98 % purity and purchased from Sigma.

Animal study

Male F344/NHsd rats (9–10 weeks of age, purchased from Harlan) were housed in solid-bottom cages with free access to food (Laboratory Chow) and water. Groups of five rats were randomly allocated to the control and treatment groups. In the first experiment, each rat was administered a single dose of vehicle (approximately 0·5 ml soya oil/rat) or SF at 150 μmol/kg body weight (freshly dissolved in the same volume of soya oil) by oral administration and was immediately transferred to metabolism cages (Tecniplast, one rat/cage) for urine collection. The control animals were killed 24 h after dosing, with 24 h urine collected and blood drawn at the end. Groups of SF-treated animals were killed at 1·5, 6 and 24 h after dosing, with urine collection for the corresponding times and blood collection when the animals were killed. Organs were promptly removed after the animals were killed and washed thoroughly under running water. In the second experiment, groups of five rats were treated with either the vehicle (approximately 0·5 ml soya oil/rat) or SF at 150 μmol/kg body weight (freshly dissolved in the same volume of soya oil) by oral administration once daily for 7 d. The animals were immediately transferred to metabolism cages after the last dose (one rat/cage) for 24 h urine collection and killed at the end for blood drawing and organ collection. All organs were thoroughly washed under running water. Plasma was prepared from each blood specimen immediately after drawing. All specimens were stored at −80°C before analysis. All protocols were approved by the Roswell Park Cancer Institute Animal Care and Use Committee.

Measurement of sulforaphane level in the plasma, tissue and urine

SF equivalents in the plasma, tissue and urine were measured by the HPLC-coupled cyclocondensation assay (reaction with 1,2-benzenedithiol), as previously described[7,30,31]. The tissues were homogenised in glass tissue grinders with five volumes of ice-cold 10 mM-Tris–HCl (pH 7·4) and 0·25 M-sucrose before analysis. In selected stomach specimens, the mucosa layer was separated from the muscle layer using a surgical knife before homogenisation, as mentioned in the Table 1 footnote. SF is metabolised through the mercapturic acid pathway *in vivo* [4]; the cyclocondensation assay detects both SF and its metabolites; the result is expressed as SF equivalents. Urinary creatinine level was measured by the creatinine assay kit from Cayman Chemical. Urinary levels of SF equivalents were presented as both μM concentrations and nmol/mg creatinine (Table 2).

Measurement of tissue glutathione S-transferase and NAD(P)H:quinone oxidoreductase 1 enzymatic activities

Tissues were homogenised in a glass homogeniser with five volumes of ice-cold 10 mM-Tris–HCl (pH 7·4) and 0·25 M-sucrose; the homogenates were then cleared by centrifugation at 12 000 ***g*** for 3 min at 4°C. Protein content was measured by the bicinchoninic acid kit from Thermo Fisher Scientific. GST activity was measured using 1-chloro-2,4-dinitrobenzene as a substrate, carried out in ninety-six-well plates as previously described[32]. NQO1 activity was also measured in ninety-six-well plates as previously described[33], with some modifications mentioned next. NQO1 catalyses menadione-mediated reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, which is measured spectroscopically. Tissue samples were diluted in 10 mM-Tris–HCl buffer (pH 7·4), and the sucrose concentration in the final assay solution was ≤1 mM (higher concentration of sucrose was found to inhibit NQO1 activity). NQO1 activity in each sample was measured in a 300-μl assay, containing 50 μl diluted sample, 50 μl phosphate buffer (5 mM-potassium phosphate, pH 7·4, containing 0·5 % dimethyl sulfoxide (DMSO)), and 200 μl assay cocktail containing 25 mM-Tris–HCl (pH 7·4), 0·2 % bovine serum albumin, 0·01 % Tween 20, 5 μM-FAD, 30 μM-NADP, 1 mM-glucose-6-phosphate, 2 U/ml glucose-6-phosphate dehydrogenase, 1·5 mg/ml 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide and 50 μM-menadione. In order to exclude non-specific enzyme activity (dicumarol non-inhibitable), 50 μl 5 mM-potassium phosphate buffer containing

0·3 mM-dicumarol and 0·5 % DMSO were added to parallel wells in place of the regular buffer. The reaction was immediately measured at 610 nm for 2 min at room temperature by a Spectra Max Plus microtitre plate reader (Molecular Devices). Specific NQO1 activity was obtained by subtracting dicumoral non-inhibitable activity from the total activity.

Statistical analyses

All results are expressed as means, with their standard errors. Difference between the means of two groups was analysed for statistical significance using the unpaired two-tailed Student's *t* test. One-way ANOVA was used for multi-group comparison, followed by Dunnett's multiple comparison test. Statistical analyses were performed using GraphPad Prism 5 software. $P<0·05$ was considered statistically significant.

Results

In rats treated with a single oral dose of vehicle (soya oil), tissue levels of SF equivalents were either very low (≤4 nmol/g tissue) or undetectable (<1 nmol/g tissue) (Table 1). The exact chemical nature of this background material is unknown, but could have been contributed by the regular diet, although SF was not present in the soya oil (data not shown). The analytical method employed in the present study (the cyclocondensation assay) can detect certain other compounds besides SF and its metabolites[30]. However, after a single oral dose of SF at 150 μmol/kg body weight (26·6 mg/kg), tissue levels of SF equivalents rapidly increased and reached up to 280 times over the background level; the highest levels were detected at 1·5 h after dosing, but SF was largely if not completely cleared from all the organs at 24 h, indicating a relatively short tissue retention time (Table 1). Among all the organs examined, exposure to SF was the highest in the stomach and the lowest in the prostate and rectum; the exposure levels between the stomach and the other two organs mentioned, at 1·5 h differed by 89- to 94-fold. Tissue exposure to SF decreased rapidly in the descending gastrointestinal tract; tissue level of SF equivalents in the stomach at 1·5 h was 6, 11, 47 and 89 times higher than that in the duodenum, jejunum, colon and rectum, respectively. Rats have both a forestomach and glandular stomach, but only the glandular stomach was analysed in the present study, as humans do not have a forestomach. Among the three genito-urinary organs, bladder exposure to SF was the highest; SF equivalents in the bladder tissue at 1·5 h were 2 and 22 times higher than in the kidney and prostate, respectively. Other organs, including the heart, liver, lung and pancreas, had very low exposure to SF, only 3–9 % of that in the stomach at 1·5 h.

In a separate experiment, rats were given oral SF (150 μmol/kg) or vehicle once daily for 7 d and killed 24 h after the last dose. Tissue levels of SF equivalents in SF-treated rats were either similar to or only slightly higher or lower than those obtained at 24 h after a single dose of SF (Table 1), indicating that repeated daily dosing of SF does not significantly elevate tissue SF levels. This finding is consistent with the result described previously showing that SF was cleared from the organs within 24 h of dosing.

Table 1. Tissue distribution of orally dosed sulforaphane (SF)* in rats
(Mean values with their standard errors)

	Tissue levels of SF equivalents (nmol/g tissue)											
	After a single SF dose								After seven daily SF doses†			
	Control		1·5 h		6 h		24 h‡		Control		SF, 24 h‡	
Organ	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
GI tract												
Stomach	2·7	0·6	818·8§	227·8	101·9	19·3	7·1	1·8	ND‖		10·2	2·6
Duodenum	2·9	1·0	144·4	36·1	24·8	5·5	4·5	0·8	1·8	0·2	4·2	0·3
Jejunum	4·0	0·9	75·1	8·2	25·5	4·5	3·2	0·3	3·5	0·8	7·9	0·3
Colon	ND		17·3	7·0	16·3	2·7	3·7	0·6	3·9	0·5	4·3	1·0
Rectum	1·4	0·4	9·2	1·0	5·6	1·0	3·5	0·5	9·6	1·1	21·2	3·9
GU tract												
Kidney	1·2	0·4	88·3	12·7	32·0	2·4	3·3	0·3	5·6	0·9	17·0	1·0
Bladder	ND		189·6	49·0	51·7	10·9	6·9	0·9	ND		6·5	0·7
Prostate	2·0	0·4	8·7	1·2	4·7	2·2	1·9	0·4	2·1	0·5	2·1	0·4
Other												
Liver	2·7	0·3	53·8	2·6	20·3	1·0	3·4	0·4	ND		3·2	0·3
Pancreas	1·9	0·6	18·7	1·7	7·9	1·2	2·9	0·4	6·1	1·0	9·1	1·0
Lung	2·4	0·4	33·5	4·0	13·7	1·7	3·3	0·5	1·0	0·4	4·9	0·4
Heart	ND		17·2	2·1	11·2	0·7	3·5	0·8	5·8	0·9	5·2	0·8

GI, gastro-intestinal; ND, not detected; GU, genito-urinary.
* SF was given to rats by oral administration at 150 μmol/kg per dose.
† Organs were removed for analysis 24 h after the last SF dose.
‡ Tissue levels of SF equivalents (after subtraction by the corresponding control values) between the two SF treatments were significantly different in the colon, heart, jejunum, kidney, liver and lung ($P<0·05$), but not in the other organs.
§ In a separate experiment, the mucosa layer and the muscle layer of a part of each stomach in this group was separated using a surgical knife and then separately analysed by the cyclocondensation assay; the levels of SF equivalents in the mucosa and muscle were 1138·0 (SEM 208·2) and 616·2 (SEM 92·2) nmol/g tissue, respectively.
‖ ND (limit of detection = 1 nmol/g tissue).

The plasma level of SF equivalents in control rats was close to zero (<0·4 μM). However, after a single oral dose of SF at 150 μmol/kg, the plasma concentration of SF equivalents increased to 15·2 μM, but decreased rapidly thereafter and was nearly undetectable at 24 h (Table 2). Thus, the plasma profile of SF equivalents closely resembles that of the tissues described previously. In contrast, urinary concentrations of SF equivalents peaked at 6 h after dosing and were 64–5509 times higher than in the plasma, reflecting remarkably high exposure of the bladder epithelium to dietary SF. Interestingly, the tissue level of SF equivalents in the bladder peaked at 1·5 h, before the urinary concentration of SF equivalents peaked. In rats given the same oral dose of SF once daily for 7 d, with 24 h urine collected following the last dose and blood collected at 24 h after the last dose, levels of SF equivalents in both plasma and urine as well as 24 h urinary recovery were almost identical to those obtained after a single SF dose (Table 2); this confirms that daily dosing of SF, for as long as a week, has no effect on its pharmacokinetic profile. Likewise, as mentioned previously, there was no clear pattern of increase in tissue levels of SF equivalents after the repeated SF treatment, compared with the single-dose treatment; some tissues showed a slight increase, including the duodenum, kidney, jejunum, liver, lung, pancreas, stomach and rectum, whereas others showed no change or a slight decrease, including the bladder, colon, heart and prostate.

We next measured GST and NQO1 activities in the rat tissues after treatment with oral SF at 150 μmol/kg once daily for 7 d, in order to assess the induction of cytoprotective phase 2 enzymes in tissue as a response to SF. As mentioned before, both enzymes play important roles in cytoprotection and cancer prevention and are known to be expressed ubiquitously and readily induced by SF. Among all the organs, the bladder showed the highest enzyme induction, with GST and NQO1 being induced 2·7- and 6·2-fold, respectively. However, the GST induction level in the stomach was not significantly different from those in the duodenum and jejunum (Fig. 1). Surprisingly, GST and NQO1 in the stomach were induced only 1·3- and 2·3-fold, respectively, which were significantly lower than those in the bladder, even though tissue exposure to SF was markedly higher in the stomach. Likewise, neither NQO1 nor GST was significantly induced in the kidney, while kidney exposure to SF was comparable to that of the jejunum, where GST and NQO1 were induced 2·2- and 2·4-fold, respectively. Among the gastro-intestinal organs, except for the stomach, the trend of enzyme induction follows that of tissue exposure to SF. Thus, induction levels of both GST and NQO1 were the highest in the duodenum (2·2- and 2·4-fold) and the lowest in the rectum (1·1- and 1·3-fold). Induction of GST and of NQO1 in the other organs, including the heart, liver, lung, pancreas and prostate were either very limited (<1·4-fold) or not detectable, correlating with low tissue exposure to SF. Overall, our results show that low tissue exposure to SF generally leads to low induction of GST and NQO1, but high tissue exposure to SF does not necessarily elicit high response of these enzymes, as was seen in the stomach and kidney.

Table 2. Plasma and urine levels of sulforaphane (SF) equivalents in SF-treated rats
(Mean values with their standard errors)

	Concentration of SF equivalents									
	Plasma (μM)			Urine					Urinary recovery as SF equivalent (% dose)	
				μM			nmol/mg creatinine†			
Oral SF at 150 μmol/kg	Mean		SEM	Mean		SEM	Mean	SEM	Mean	SEM
After a single dose										
Control		ND*			ND			ND		
1·5 h	15·2		3·9	974·5		755·4	1154·2	444·7	4·0	1·1
6 h	5·6		0·9	4390·6		1078·1	2745·2	792·9	22·6	5·5
24 h	0·5[a]		0·2	2784·3[b]		640·4	2633·3[c]	508·2	60·8[d]	6·5
Daily dose for 7 d‡										
Control		ND			ND			ND		
24 h	0·7[a]		0·4	2646·5[b]		596·7	2461·2[c]	710·1	57·3[d]	7·7

ND, not detected.
[a,b,c,d]Mean values with same superscript letters were not significantly different.
* ND (the detection of limit for plasma specimen and urine specimen was 0·5 and 0·2 μM, respectively).
†Urinary creatinine levels: 1·4 (SEM 0·1) mg/ml.
‡24 h urine was collected after the last SF dose, with the blood drawn at the end.

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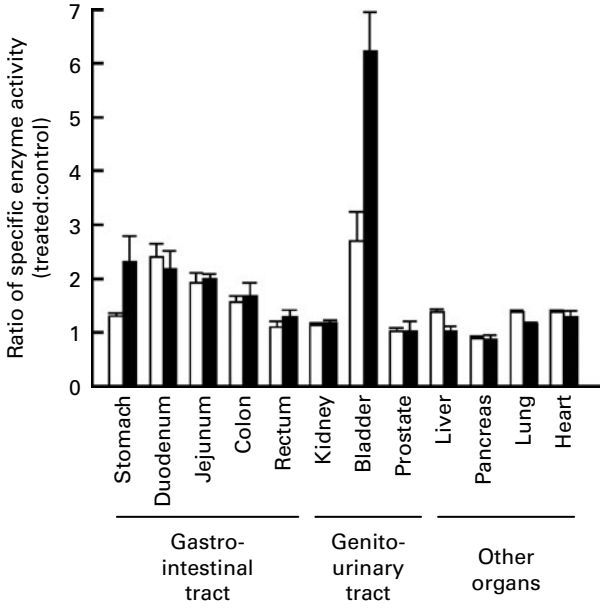

Fig. 1. Induction of glutathione *S*-transferase (GST, □) and NAD(P)H:quinone oxidoreductase 1 (NQO1, ■) by dietary sulforaphane (SF) in rat tissues *in vivo*. Groups of five male F344 rats were given vehicle (soya oil) or SF (150 μmol/kg) by oral administration once daily for 7 d. The animals were killed 24 h after the last dose. Tissue enzymatic activities of GST (1-chloro-2,4-dinitrobenzene as a substrate) and NQO1 (menadione-mediated and dicumoral-inhibitable 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reduction) were measured and calculated as relative specific enzyme activity. Values are means, with their standard errors represented by vertical bars. The specific GST activity (pmol/min per mg protein) in the control tissues ranged from 12·1 (SEM 2·9) in the heart to 352·9 (SEM 58·0) in the liver. The specific NQO1 activity (pmol/min per mg protein) in the control tissues ranged from 2·6 (SEM 0·8) in the pancreas to 181·4 (SEM 70·5) in the stomach. The NQO1 value in the bladder was significantly different from those in any other organ. The GST value in the bladder was not significantly different from those in the duodenum, jejunum and colon, but significantly different from those in other organs.

Discussion

The present study shows that 61 % of the SF dose is recovered in the urine in F344 rats within 24 h of oral administration (Table 2). This value is lower than the 72–75 % recovery shown previously in 24 h urine of Sprague–Dawley rats dosed with SF either in pure form or via broccoli sprout extracts[7,26,29]. Whether animal strain accounts for the difference remains unclear, but in human subjects given a broccoli sprout preparation, 70 % of the SF dose was recovered in approximately 12 h[25]. Collectively, these data show that dietary SF has a very high bioavailability but it is also rapidly eliminated through urinary excretion. The rapid elimination of SF, also reflected in the rapid decline of plasma and tissue levels of SF equivalents, is consistent with our finding that daily dosing of SF for 7 d did not significantly alter the levels of SF equivalents in the plasma, tissues and urine. Most, if not all, SF equivalents measured in the urine are probably the *N*-acetylcysteine conjugate, as it was previously shown that 72 % of a single oral dose of SF was detected as such in 24 h urine in Sprague–Dawley rats[26]. However, in human subjects dosed with SF using broccoli sprout extracts, 65 and 28 % of urinary SF equivalents were the *N*-acetylcysteine conjugate and cysteine conjugate, respectively[34]. The cysteine conjugate is the precursor of the *N*-acetylcysteine conjugate in the mercapturic acid pathway. Although the chemical identity of the SF equivalents present in the rat plasma remains undefined, Gasper *et al.*[35] reported that in the plasma of human volunteers taking SF via broccoli, more than 40 % of SF equivalents were SF itself, approximately 30 % as the cysteine conjugate, and less than 10 % as the *N*-acetylcysteine conjugate[35]. These values should be viewed with caution, however, because of the instability of these compounds, as will be discussed next.

In the present study, the cyclocondensation assay was used to measure both SF and all its metabolites formed in the mercapturic acid pathway (expressed as SF equivalents). It was previously shown that the SF metabolites formed in the mercapturic acid pathway are unstable and readily dissociate back to SF (see Introduction section, for further information). In view of the instability of these metabolites, we have avoided measuring individual metabolites, because the result of such measurement may not reflect their true levels *in vivo*. On the other hand, these metabolites are widely considered as SF carriers. Many studies have shown that the chemopreventive activity of the *N*-acetylcysteine conjugate of SF is very similar to that of SF, including induction of NQO1 in murine hepatoma cells[36], inhibition of proliferation and induction of apoptosis in human bladder cancer cells and human prostate cancer cells[37,38], inhibition of malignant progression of lung adenomas induced by tobacco carcinogens in mice[10], and inhibition of colonic aberrant crypt foci in rats[9].

A striking result from the present study is that the urinary concentrations of SF equivalents at 1·5, 6 and 24 h post SF dosing are 64-, 784- and 5509-fold higher than the corresponding plasma concentrations, respectively (Table 2). This probably explains why bladder tissue uptake of dietary SF is profoundly higher than that of tissues to which dietary SF is delivered through blood circulation, such as heart, liver, lung, pancreas and prostate. Consistent with this finding, induction of GST and NQO1 in rats treated with SF was markedly higher in the bladder (2·7-fold for GST and 6·2-fold for NQO1) than in the other organs mentioned previously (≤ 1·4-fold induction of each enzyme) (Fig. 1). This suggests that, if induction of GST and NQO1 plays an important role in the chemopreventive activity of SF, its protective effect should be greatest in the bladder. Mammary gland exposure and response to dietary SF could not be measured due to the use of male rats, but may not differ significantly from those of the heart, liver, lung, pancreas and prostate, as delivery of SF to the mammary gland also undoubtedly relies on its transport through the blood circulatory system. Tissue levels of SF equivalents in the stomach were the highest among all organs analysed, but rapidly decreased in the descending gastro-intestinal tract, and were almost undetectable in the rectum. Such a tissue accumulation pattern of SF may largely reflect the net effect of direct exposure to ingested SF and rapid absorption of SF into the blood through the gastro-intestinal tract. High exposure to SF in the stomach did not result in correspondingly high enzyme induction. SF level in the stomach was markedly higher than in the bladder, but induction of GST and NQO1

was much more significant in the latter ($P<0·05$). SF level in the stomach tissue was also markedly higher than that of the duodenum, but GST induction was significantly higher in the latter ($P<0·05$), and NQO1 induction levels were virtually the same in the two organs. Notably, SF was able to effectively penetrate into the stomach tissue, as levels of SF equivalents in the mucosa layer and the muscle layer of the stomach, 1·5 h after rats were given SF, were 3·3- and 6·0-fold higher than that in the bladder, respectively (see Table 1). The reason for high gastric uptake of SF but low induction of GST and NQO1 remains unexplained. In the other gastro-intestinal organs, the rank order of enzyme induction levels correlated with the rank order of tissue exposure to SF. Enzyme induction in the kidney also did not reflect tissue exposure to SF. Kidney exposure to SF was comparable to that of the jejunum, but neither GST nor NQO1 was significantly induced in the kidney, whereas each enzyme was induced 2-fold in the duodenum. Collectively, our study results show that tissue exposure to dietary SF is profoundly different among different organs, and that while induction of the phase 2 enzymes is low in organs with low exposure to SF, high exposure to SF does not necessarily lead to high enzyme induction, as in the stomach and kidney. To be sure, induction of the phase 2 enzymes is only one of the many chemopreventive properties of SF[4]; further study is needed to assess the relationship between tissue exposure to SF and other chemopreventive effects of this agent.

In the present study, the animals were given SF by oral administration and then immediately moved to metabolism cages for urine collection. The food matrix effect on GST and NQO1 induction by SF could not be examined due to urine contamination by the diet kept in the metabolism cage. However, a potential matrix effect (both plant matrix and food matrix) on GST and NQO1 induction by SF was addressed in our previous study[29]. We showed that induction of both GST and NQO1 in cultured cells by an SF-containing broccoli sprout extract was comparable to that of pure SF on the basis of SF concentrations. We also showed that bladder induction levels of both GST and NQO1 in rats *in vivo* were 1·5- to 2-fold higher when the SF-containing broccoli sprout extract was given by oral administration than those obtained when the same amount of extract was fed to the animals in the diet.

SF is currently widely investigated as a chemopreventive agent. Preclinical and clinical studies of SF have been carried out in a variety of organs, as mentioned before. Our present study shows that dietary SF reaches different organs in vastly different concentrations and that the chemopreventive activity of dietary SF may differ significantly among organs. The data presented in this report may provide a basis for prioritising organs for further chemopreventive study of SF. Given the relatively high tissue uptake of dietary SF by the bladder, apparently due to high urinary concentration of SF metabolites, and the strong response of tissue GST and NQO1 to SF, this organ seems to be particularly relevant for further SF study. Indeed, many lines of evidence indicate that SF is a highly promising cancer chemopreventive agent in the bladder. SF was recently shown to inhibit DNA damage in bladder cells and tissues induced by 4-aminobiphenyl, a major human bladder carcinogen from tobacco smoke and other environmental sources[39]. SF inhibits the growth of human bladder cancer cells[40], and an SF-enriched broccoli sprout extract inhibits bladder cancer development in a rat model[7]. Moreover, several epidemiological studies have shown an inverse association between broccoli consumption and human bladder cancer risk and survival[41–43].

Tissue uptake of dietary SF was by far the highest in the stomach among all the organs examined, apparently due to direct exposure to ingested SF, although induction of GST and NQO1 in this organ was relatively modest. A number of studies have shown that SF or SF-rich broccoli sprouts kill *Helicobacter pylori*, a major cause of gastritis and stomach cancer, and inhibit colonisation and gastritis in *H. pylori*-infected mice and human subjects[15,23,44]. Our present data provide support for targeting stomach *H. pylori* with SF, although it remains to be shown if SF retention in the stomach after oral dosing is sufficiently long to kill the bacteria there.

Our data suggest that potential overdosing or toxicity in the bladder and the upper gastro-intestinal tract is possible, should high doses of SF be used for chemoprevention in other organs. SF is a reactive electrophile, and can inhibit cell proliferation and cause cell death at relatively high concentrations[4]. In this context, it is worth noting that animal studies that showed anticancer efficacy in organs such as the colon, lung, mammary gland and prostate were often conducted with high doses of SF ranging from 225 to 600 μmol/kg[2,8,10,15,18,19]; under these dosing conditions, the stomach as well as the bladder could be exposed to extremely high levels of SF. In the present study, urine concentrations of SF equivalents reached up to 4·39 mM in rats dosed with SF at 150 μmol/kg, but no bladder toxicity was detected. The normal bladder epithelium is equipped with several protective barriers, including tight junction, thickened apical membrane and coverage by a mucopolysaccharide[45]. It is not known at the present time what is the maximally tolerated non-toxic dose for SF, but it is conceivable that chronic presence of very high urinary concentrations of SF and its metabolites may be harmful to the bladder. In cultured bladder cell lines, SF and its *N*-acetylcysteine conjugate could cause cell growth inhibition, apoptosis and cell cycle arrest at micromolar concentrations[38,40].

SF is one of the naturally occurring isothiocyanates that show promising cancer preventive activity. Several other isothiocyanates, including allyl isothiocyanate, benzyl isothiocyanate and phenethyl isothiocyanate have also been widely studied as cancer chemopreventive agents. All these compounds are metabolised through the mercapturic acid pathway and disposed in urine, suggesting that tissue exposure and response to these compounds may resemble that of SF. Their cancer chemopreventive activities resulting from dietary intake may also vary significantly among organs.

Acknowledgements

The authors thank Joseph D. Paonessa for valuable technical assistance. The present work was supported in part by US

NIH/NCI grant R01CA124627. The authors confirm that there are no conflicts of interest. J. R. M. and Y. Z. secured the funding for the research. O. L. V., A. B., J. R. M. and Y. Z. designed the study. O. L. V., A. B. and Y. Z. conducted the study and data analysis. Y. Z. wrote the manuscript. All authors read, edited and approved the manuscript.

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