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Induction of glutathione *S*-transferase activity in hepG2 cells by extracts from fruits and vegetables

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The human hepatoma cell line, hepG2, retains many of the xenobiotic metabolising enzymes found in normal hepatocytes, including an inducible glutathione *S*-transferase (GST). The isoform of GST that is induced by xenobiotics in this cell line is GSTA1-1. As a first step to determining the effect of diet on induction of GST in humans, we have examined the ability of extracts from a wide variety of fruits and vegetables to induce GST activity in hepG2 cells. Extracts from cruciferous vegetables (broccoli, Brussels sprouts, cabbage) were the most potent inducers, but this was dependent on the variety. Most of the extracts from fruits, with the exception of grapefruit, were poor inducers. Similarities and differences between the induction of GST and of quinone reductase in mouse hepa1c1c7 cells are discussed. The results show that extracts from cruciferous vegetables are effective inducers of human GST, in agreement with previous studies on GST in animals and cell lines derived from animals. © 1997 Elsevier Science Ltd

INTRODUCTION

There is now very convincing epidemiological evidence to show that a diet high in fruit and vegetables protects against chronic diseases such as cancer and heart disease (Block *et al.*, 1992). In particular, consumption of cruciferous vegetables was particularly protective towards cancers of the colon and thyroid (Steinmetz & Potter, 1991). A substantial part of this protective effect of cruciferous vegetables has been ascribed to the induction of antioxidant and detoxifying enzyme defences (phase II enzymes), mainly in the gut, liver and kidneys of experimental animals (Nijhoff *et al.*, 1993; Bradfield *et al.*, 1985). This induction of phase II enzymes, especially glutathione *S*-transferase (GST, EC 2.5.1.18), which detoxifies xenobiotics and potential carcinogens by conjugation with glutathione, was closely correlated to reduction in the number of chemically induced tumours in rodents (Wattenberg, 1983, 1985). However, very few studies have addressed induction of these enzymes by food components in humans or human cells. Apart from one study by Bogaards *et al.* (1994), which demonstrated that a diet high in Brussels sprouts induced plasma GSTα twofold in humans, it is not known which fruit or vegetables are the best inducers in human systems. In this study, we have used a highly differentiated cell line (hepG2) which not only resembles morphologically normal hepatocytes (Bouma *et al.*, 1989) but has also been shown to retain many of the enzymes involved in xenobiotic metabolism, including a functional Ah receptor (Roberts *et al.*, 1990), an inducible UDP-glucuronosyl transferase (Grant *et al.*, 1988), an inducible sulphotransferase (Dawson *et al.*, 1985) and an inducible quinone reductase (QR, EC 1.6.99.2; also called NAD(P)H quinone oxidoreductase$_1$ or DT diaphorase) (Backman *et al.*, 1991). In this cell line, GST is also inducible, and the predominant isoform present in control cells is alpha (A1-1) (Lewis *et al.*, 1989; Castro *et al.*, 1990). A1-1 is also the isoform that is induced in this cell line (Dierickx, 1994). Importantly, this isoform of GST is able to detoxify xenobiotics and also to protect against free radical mediated damage (Mannervik & Danielson, 1988). We have demonstrated that many extracts from cruciferous vegetables are able to induce GST activity in human hepG2 cells.

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MATERIALS AND METHODS

Materials

Biochemicals and cell culture reagents were from Sigma Chemical Co. or from Imperial. HepG2 cells were obtained from The European Cell Culture Collection.

Preparation of extracts

Extracts from fruit and vegetables were prepared as previously described (Tawfiq *et al.*, 1994). Briefly, the method consists of freeze-drying fresh samples of fruit and vegetables, followed by grinding each sample to a

fine powder. For samples labelled as raw, 70% methanol was added, followed by gentle boiling for 15 min. The methanol was removed by rotary evaporation followed by adjustment to a fixed volume with water. The resulting extract contains no methanol and the procedure maximises extraction of the aqueous components of the fruit and vegetables. Samples that were classified as autolysed were treated as follows: water instead of methanol was added to the dried powder, allowed to stand for 1 h at room temperature, and then methanol added and the sample extracted as described above. Samples that were classified as cooked were boiled in water for 15 min before freeze-drying and treatment as described above for raw extracts. All extracts were frozen until required. Before addition to the cell culture, all were filtered through a 0.22 μm filter. The concentrations shown are expressed as dry weight of original fruit or vegetable per millilitre of culture medium.

Cell culture

hepG2 cells were grown in Eagle's minimal essential medium, supplemented with glutamine (2 mM), penicillin (50 units ml^{-1}), streptomycin (50 μg ml^{-1}) and foetal calf serum (10%). Cultures of 10 ml were incubated in CO_2 (5%) at 37°C in a humidified incubator and split 1:6 (using trypsin (0.25%) and EDTA (0.02%)) every 7 days. Under these conditions, the cell cycle is about 30 h. The medium was changed in stock cultures every 3 days. Food extracts were added (up to 400 μl per 10 ml of cell culture medium) for 24 h. After this time, cells were removed from dishes using trypsin/EDTA, treated with trypsin inhibitor for 5 min, then washed twice with Dulbecco's phosphate buffered saline (10%) (PBS). Cells were counted after a third PBS wash using a haemocytometer slide. Cells were suspended in 0.1% digitonin/0.05 M phenylmethylsulphonyl fluoride in 0.02 M phosphate (1 ml), mixed thoroughly and then extracted using a sonic bath in ice for two periods of 15 min. Cells were demonstrated to be lysed using trypan blue exclusion. Cell extracts were centrifuged at 10000*g* for 5 min, and used to determine GST activity.

Assay of glutathione *S*-transferase activity

Assays were performed using glutathione and 1-chloro-2,4-dinitrobenzene (CDNB) (Habig & Jakoby, 1981) in

Table 1. Induction of GST activity in hepG2 cells and of QR in hepa1c1c7 cells by extracts from fruit and vegetables

Material	Variety and method[b]	GST activity (U mg^{-1})	Induction of GST (-fold)	Induction of QR[a] (-fold)
Broccoli	A	0.13	1.4*	1.5*
Broccoli	R	0.11	1.2	1.4*
Broccoli	Marathon, R	0.12	1.4*	ND
Brussels sprouts	Edmund, A	0.15	1.7*	1.5*
Brussels sprouts	Edmund, C	0.16	1.8*	1.3*
Brussels sprouts	Edmund, R	0.13	1.4*	NI
Brussels sprouts	Content, A	0.15	1.7*	1.3*
Brussels sprouts	Content, R	0.13	1.4*	1.1*
Brussels sprouts	Ottoline, A	0.12	1.4*	1.2*
Cabbage	Savoy Rhapsody, A	0.12	1.4*	1.3*
Cabbage	Savoy Rhapsody, C	0.14	1.6*	NI
Cabbage	White, R	0.09	1.0	1.3*
Cauliflower	White Rock, A	0.10	1.1	1.4*
Cauliflower	White Rock, C	0.12	1.4*	1.1*
Cauliflower	White Rock, R	0.09	1.0	NI
Cauliflower	Limelight, A	0.15	1.7*	1.1*
Red cabbage	Autoro, A	0.12	1.4*	1.2*
Red cabbage	Autoro, R	0.09	1.0	NI
Red Cabbage	Autoro,C	0.12	1.4*	ND
White cabbage	Slawdena, R	0.14	1.6*	1.2*
Grapefruit	A	0.13	1.4*	1.1*
Petit pois	R	0.08	0.9	1.2*
Petit pois	C	0.07	0.8	1.4*
Green pepper	A	0.10	1.1	1.2*
Garden peas	R	0.10	1.1	1.3*
Garden peas	C	0.09	1.0	1.3*
Garden peas	A	0.09	1.0	1.1*
Red pepper	R	0.07	0.8	1.3*
Lettuce	Lollo rosso, A	0.12	1.4	1.3*
Lettuce	Lollo rosso, R	0.09	1.0	1.2

[a]Data from Tawfiq *et al.* (1994), with the concentration of extract at 2.1 mg dry weight ml^{-1} culture medium.
[b]A, autolysed; C, cooked; R, raw. Concentration of extract used to test for GST induction in each case was 1.3 mg dry weight/ml of culture medium.
*Indicates inducer of GST or QR activity. The following extracts were tested but did not induce either GST activity in hepG2 cells or QR activity in hepa1c1c7 cells: Lemon (autolysed); carrots (Bangor; raw, cooked and autolysed); potato (King Edward, cooked); apple (Cox; raw and autolysed); green pepper (raw); red pepper (raw); plums (raw); tomatoes (Canary; autolysed). No induction of GST activity was observed with (not tested in QR induction assay): green grapes (raw); black cherries (raw); blackcurrants (raw); parsnips (new white skin; cooked).
NI, no induction; ND, not determined.

the presence of Triton X-100 (0.04%) at 30°C, using an extinction coefficient of 9600 M^{-1} cm^{-1} at 340 nm. Assays were performed at least in triplicate on each sample. Protein was assayed using the method of Lowry *et al.* (1951) using bovine serum albumin as a standard.

RESULTS

The number of hepG2 cells after 24 h of growth was estimated as $1.01\times10^7\pm0.26\times10^7$ (mean and standard deviation of 97 determinations) in 10 ml of culture medium. After lysis, these cells, originally from 10 ml of culture medium, yielded 2.02 ± 0.46 mg of protein. The level of GST was measured using CDNB as substrate. The amount of GST in these baseline cells was 0.090 ± 0.011 units mg^{-1} protein, which compares to previous reports on this cell line (0.00672 ± 0.0002 units mg^{-1} protein for cells grown in Dulbecco's modified Eagle's medium (Doostdar *et al.*, 1988), 0.014 units mg^{-1} protein (Castro *et al.*, 1990), $0.045\pm$ 0.0028 units mg^{-1} protein (Dierickx, 1994), 0.075 units mg^{-1} protein (Lewis *et al.*, 1989) and 0.112 ± 0.004 units mg^{-1} protein (Dierickx, 1989). The content of GST activity, as estimated using CDNB, is comparable to that in freshly isolated adult human hepatocytes (Duthie *et al.*, 1988).

After addition of extracts (0, 0.32, 0.65, 1.3 mg dry weight ml^{-1} of cell culture medium) from autolysed broccoli followed by incubation for 24 h, the activity of GST in hepG2 cells increased by 1-, 1.2-, 1.3- and 1.4-fold respectively. Extracts from other fruits and vegetables were also tested at a concentration of 1.3 mg dry weight ml^{-1} of culture medium (Table 1). Significant induction using food extracts was observed at values of 1.4-fold induction or greater, and extracts which may be potential inducers of human GST activity are indicated in the table. It is clear that most of the crucifers contain compounds that are able to induce GST activity in these cells. For comparison, the ability of these extracts to induce QR in hepa1c1c7 cells (Tawfiq *et al.*, 1994), using an established and validated assay (Prochaska *et al.*, 1992), is also shown. Most of the extracts from fruits were unable to induce GST activity, with the exception of grapefruit.

DISCUSSION

The data presented in this paper indicate that certain extracts from cruciferous vegetables induce GST activity in human hepG2 cells. There is evidence to show that, at least in some species, there is some coordinate induction of GST and QR (Johnson *et al.*, 1994). We have compared our data on human GST with that of mouse QR in the hepa1c1c7 cell line. There is a clear trend in that cruciferous vegetables are often effective inducers in both systems, but, in 38 extracts which can be compared, 13 were inducers in both systems, 13 were inducers in neither system, 11 induced QR but not GST activity, and one induced GST but not QR activity. This supports previous evidence (reviewed in Johnson *et al.*, 1994) that, although many compounds induce both GST and QR in several species so far tested, there are some marked differences between the two enzymes. Furthermore, there is evidence that QR and GSTα are not coordinately induced in human cells (Eickelmann *et al.*, 1994).

It has already been established that GSTα is induced in animal models and in cell lines derived from animals, especially rodents (see Johnson *et al.*, 1994). The 5′ flanking region of the rat and mouse GSTα genes contains an antioxidant responsive element (ARE) (TGACNNNGCA) which is thought to be responsible for mediating induction by xenobiotics and dietary compounds (Rushmore *et al.*, 1991). However, the 5′-flanking sequence of the human GSTα genes (both A1 and A2) did not contain any ARE sequences (Morel *et al.*, 1994) and, indeed, the full 5′-flanking region linked to a reporter did not show inducibility in transfection experiments (Suzuki *et al.*, 1994). Therefore, since stimulation of GST by diet plays such an important role in protecting animals against chemically induced carcinogenesis, it is vitally important in assessing chemoprevention in humans to determine if GSTα is also inducible in man by diet. The preliminary study presented here demonstrates that human GST activity is inducible by extracts from normal foods, especially crucifers. From other studies on the induction of GST in hepG2 cells, the isoform that is induced is most likely to be the A1-1 form (Dierickx, 1994). However, it should be noted that the activity measurements presented here are intended as a screening assay for inducers of human GST activity, and there is no reason to expect that the magnitude of induction reflects that *in vivo*, which on the basis of one study may be higher (Bogaards *et al.*, 1994). In summary, the system presented here is intended to indicate which foods are inducers of GST activity in humans to facilitate design of future experiments.

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