



Antiproliferative and antioxidant activities of common vegetables: A comparative study

Dominique Boivin^a, Sylvie Lamy^a, Simon Lord-Dufour^a, Jessica Jackson^a, Edith Beaulieu^a, Martine Côté^c, Albert Moghrabi^b, Stéphane Barrette^b, Denis Gingras^a, Richard Béliveau^{a,b,*,1}

^aLaboratoire de Médecine Moléculaire, Centre de Cancérologie Charles-Bruneau, Hôpital Ste-Justine, Université du Québec à Montréal, 3175 Côte Ste-Catherine, Montréal, Québec, Canada H3T 1C5

^bService d'hématologie-oncologie, Centre de Cancérologie Charles-Bruneau, Hôpital Ste-Justine, 3175 Chemin Côte-Ste-Catherine, Montréal, Québec, Canada H3T 1C5

^cMinistère de l'Agriculture, des Pêcheries et de l'Alimentation du Québec, 200, chemin Sainte-Foy, Québec, Canada G1R 4X6

ARTICLE INFO

Article history:

Received 12 February 2008

Received in revised form 5 May 2008

Accepted 23 May 2008

Keywords:

Cancer prevention
Cruciferous vegetables
Allium vegetables
Antioxidants

ABSTRACT

Epidemiological studies have consistently linked abundant consumption of fruits and vegetables to a reduction of the risk of developing several types of cancer. In most cases, however, the identification of specific fruits and vegetables that are responsible for these effects is still lacking, retarding the implementation of effective dietary-based chemopreventive approaches. As a first step towards the identification of foods endowed with the most potent chemopreventive activities, we evaluated the inhibitory effects of extracts isolated from 34 vegetables on the proliferation of 8 different tumour cell lines. The extracts from cruciferous vegetables as well as those from vegetables of the genus *Allium* inhibited the proliferation of all tested cancer cell lines whereas extracts from vegetables most commonly consumed in Western countries were much less effective. The antiproliferative effect of vegetables was specific to cells of cancerous origin and was found to be largely independent of their antioxidant properties. These results thus indicate that vegetables have very different inhibitory activities towards cancer cells and that the inclusion of cruciferous and *Allium* vegetables in the diet is essential for effective dietary-based chemopreventive strategies.

© 2008 Elsevier Ltd. All rights reserved.

1. Introduction

It is currently estimated that dietary factors account for approximately one third of cancer death, similar to the impact of smoking (Doll & Peto, 1981). Such a close relationship between diet and cancer is well illustrated by the large variations in rates of specific cancers among countries and by the observations that these rates are strongly correlated with differences in several aspects of the diet (Doll & Peto, 1981; Willett, 2002). Among the dietary factors that are most closely linked to cancer, a large number of population-based studies have consistently shown that individuals who eat five servings or more of fruits and vegetables daily have approximately half the risk of developing a wide variety of cancer types, particularly those of the gastrointestinal tract (Gescher, Pastorino, Plummer, & Manson, 1998; World Cancer Research Fund & Amer-

Abbreviations: ORAC, oxygen radical absorbance capacity

* Corresponding author. Address: Laboratoire de Médecine Moléculaire, Centre de Cancérologie Charles-Bruneau, Hôpital Ste-Justine, 3175 Côte Ste-Catherine, Montréal, Québec, Canada H3T 1C5. Tel.: +1 514 345 2366; fax: +1 514 345 2359.

E-mail address: molmed@recherche-ste-justine.qc.ca (R. Béliveau).

¹ Holder of the "Chaire en prévention et traitement du cancer" from UQAM and of the "Chaire Claude-Bertrand en Neurochirurgie" from Université de Montréal.

ican Institute for Cancer Research, 1997). These chemopreventive properties of fruits and vegetables arise from their high content of phytochemicals such as phenolic compounds (Naczek & Shahidi, 2004, 2006) that target several key events involved in the development of cancer (Dorai & Aggarwal, 2004; Surh, 2003). Potential mechanisms for cancer prevention of phytochemicals include prevention of DNA adduct formation (Ames, Gold, & Willett, 1995), enhanced carcinogen elimination (Talalay, 2000), inhibition of inflammatory processes (Surh et al., 2001), interference with tumour angiogenesis (Lamy, Gingras, & Béliveau, 2002; Tosetti, Ferrari, De Flora, & Albini, 2002), as well as through a direct cytotoxic effect on tumour cells (Martin, 2006). This pleiotropic mechanism of action of phytochemicals imply that the chemopreventive properties that are associated with fruits and vegetables consumption are complex and likely arise from synergistic combinations from several distinct molecules, not only within a given food but also from the overall composition of the diet (Lee, Lee, & Lee, 2004; Liu, 2003; McCullough & Giovannucci, 2004). Clearly, the identification of specific foods or food groups that have beneficial effects on certain types of cancer represent an important issue in order to bonify current chemopreventive strategies based on increased consumption of fruits and vegetables.

As a first step towards this goal, we have undertaken a systematic evaluation of the chemopreventive potential of several vegetables by monitoring their antiproliferative effects on a wide variety of tumour cell lines. Surprisingly, we found that there are considerable differences in the ability of vegetables to inhibit the proliferation of various cancer cells. We observed that this anticancer property is preferentially associated with cruciferous and *Allium* vegetables, whereas most vegetables commonly consumed in Western countries have a much weaker antiproliferative effects. These results illustrate the need to improve current dietary recommendations by actively promoting increased consumption of cruciferous and *Allium* vegetables as an essential means to reduce the incidence of cancer.

2. Materials and methods

2.1. Chemicals

Fluorescein sodium salt, 2,2'-azobis (2-methylpropionamide) dihydrochloride (AAPH) and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) were purchased from Sigma-Aldrich (Oakville, ON, Canada).

2.2. Preparation of vegetable juices

Fresh vegetables were obtained from local producers (Montreal, QC, Canada) in May–July 2005, stored at 4 °C, and processed within 24 h. Juices were prepared by passing 100 g of vegetables through a domestic centrifugal juice extractor (Juiceman Professional series 210, model JM210C, Montreal, QC, Canada). The liquid obtained was clarified by centrifugation at 50,000g, 45 min at 4 °C. The supernatant was then sterilized by filtration through a 0.22- μ m filter, and aliquots were immediately frozen in liquid nitrogen. Protein concentrations were determined by the Bradford method using the Coomassie Plus assay kit (Pierce).

2.3. Cell culture

All cell lines were cultured at 37 °C under a humidified atmosphere containing 5% CO₂. AGS (stomach adenocarcinoma, ATCC CRL-1739) were cultured in F12-K medium containing 10% FBS; MCF-7 (mammary gland adenocarcinoma, ATCC HTB-22) were cultured in MEM containing 0.01 mg/ml insulin and 10% FBS; Panc-1 (pancreatic carcinoma ATCC CRL-1469) were cultured in DMEM high glucose containing 10% FBS; PC-3 (prostatic adenocarcinoma, ATCC CRL-1435) were cultured in Ham's F12 containing 10% calf serum; A549 (lung carcinoma, ATCC CCL-185) cells were cultured in DMEM low glucose containing 10% calf serum; Daoy (medulloblastoma, ATCC HTB-186) and U-87 MG (glioblastoma, ATCC HTB-14) cells were cultured in MEM containing 10% FBS; Caki-2 (renal carcinoma, ATCC HTB-47) cells were cultured in McCoy's 5A medium containing 10% FBS. NHDF (normal human dermal fibroblasts) were cultured in FGM-2 medium (Clonetics) containing 1 ng/ml hFGF, 5 μ g/ml insulin, and 2% FBS.

2.4. Cell proliferation assay

Cells were plated in 96-well plates at 2500–5000 cells/well in 200 μ l complete medium and incubated at 37 °C under a humidified atmosphere containing 5% CO₂ for 24 h. The next day, the medium was removed and replaced by 100 μ l fresh medium containing 1% FCS and the specified concentrations of juices. Cell viability was determined by assaying the mitochondrial activity of treated cells after a 48 h incubation, with the highly sensitive WST-1 assay. Briefly, 10 μ l of tetrazolium salt WST-1 reagent was added to each

well and the soluble formazan dye produced by metabolically active cells was monitored every minute for 30 min at 37 °C on a SpectraMax Plus reader (molecular devices).

2.5. Oxygen radical absorbance capacity (ORAC) assay

The ORAC-fluorescein assay was performed essentially as described previously (Dávalos, Gómez-Cordovés, & Bartolomé, 2004) with minor modifications. Briefly, 20 μ l of antioxidant (vegetable extracts or Trolox standards), and 120 μ l of 0.117 μ M fluorescein in 75 mM phosphate buffer, pH 7.4 were pipetted into the well of the microplate. The mixture was preincubated for 15 min at 37 °C, and then 60 μ l of 40 mM AAPH were added rapidly using an electronic multichannel pipette. The fluorescence ($\lambda_{\text{ex}} = 485$ nm; $\lambda_{\text{em}} = 520$ nm) was recorded every min for 80 min using a SpectraMAX™ Gemini fluorescence plate reader (Molecular Devices). Calibration solutions of Trolox (0.5–8 μ M) were also carried out in each assay. Data were exported from the SoftMax Pro 3.1 software to Excel (Microsoft) for further calculations. The area under the fluorescence decay curve (AUC) was calculated as $AUC = 1 + f_1/f_0 + f_2/f_0 + \dots + f_{80}/f_0$ where f_0 is the initial fluorescence at $t = 0$ and f_i the fluorescence at $t = i$. ORAC-FL values were expressed as Trolox equivalents by using a standard curve and regression analysis performed using the Prism 4.0 software (GraphPad Software, San Diego, CA).

3. Results

3.1. Inhibition of tumour cell proliferation by vegetable extracts

As a first step towards the identification of vegetables containing antiproliferative activities towards cancer cells, the inhibitory effects of extracts from a wide variety of commonly consumed vegetables on eight different tumour cell lines derived from stomach, kidney, prostate, breast, brain, pancreatic and lung cancer were examined. There was considerable differences in the sensitivity of these cell lines to the vegetable extracts (Fig. 1). Tumour cells derived from prostate and stomach cancer were most sensitive to the extracts while cells from kidney, pancreatic and lung cancers were much less affected by the tested extracts. For example, 23 of the 34 the tested vegetable extracts inhibited the proliferation of prostate tumour cells by more than 50%, while only 7 extracts were active against kidney cancer cells (Table 1).

In addition to cruciferous vegetables, all members of the *Allium* family tested in this study were powerful inhibitors of tumour cell proliferation. In fact, among all vegetables tested in this study, the extract from garlic was by far the strongest inhibitor of tumour cell proliferation, with complete growth inhibition of all tested cell lines. Leek, immature (green) and mature (yellow) onions were also highly inhibitory against most cell lines, although green onion was less active against tumour cells from the kidney, while yellow onion was a modest inhibitor of the lung tumour cells and had no significant inhibitory activity against kidney tumour cells. Overall, these results indicate that there is substantial differences in the antiproliferative properties of vegetables against tumour cells and that cruciferous, dark green and *Allium* vegetables are endowed with potent anticancer properties (Table 2).

The potency of inhibition of cell proliferation by cruciferous and *Allium* vegetables was then investigated, using serial dilutions of the extracts (Fig. 2a). Garlic was the most potent inhibitor of cell proliferation with a marked reduction of U-87 glioblastoma cell proliferation at a 1/1000 (corresponding to 3.32 mg raw vegetable/ml) dilution of the extract. Brussels sprouts extracts also strongly inhibited the proliferation of these cells, with 30% inhibition at a 1/1000 dilution (3.32 mg raw vegetable/ml) and complete

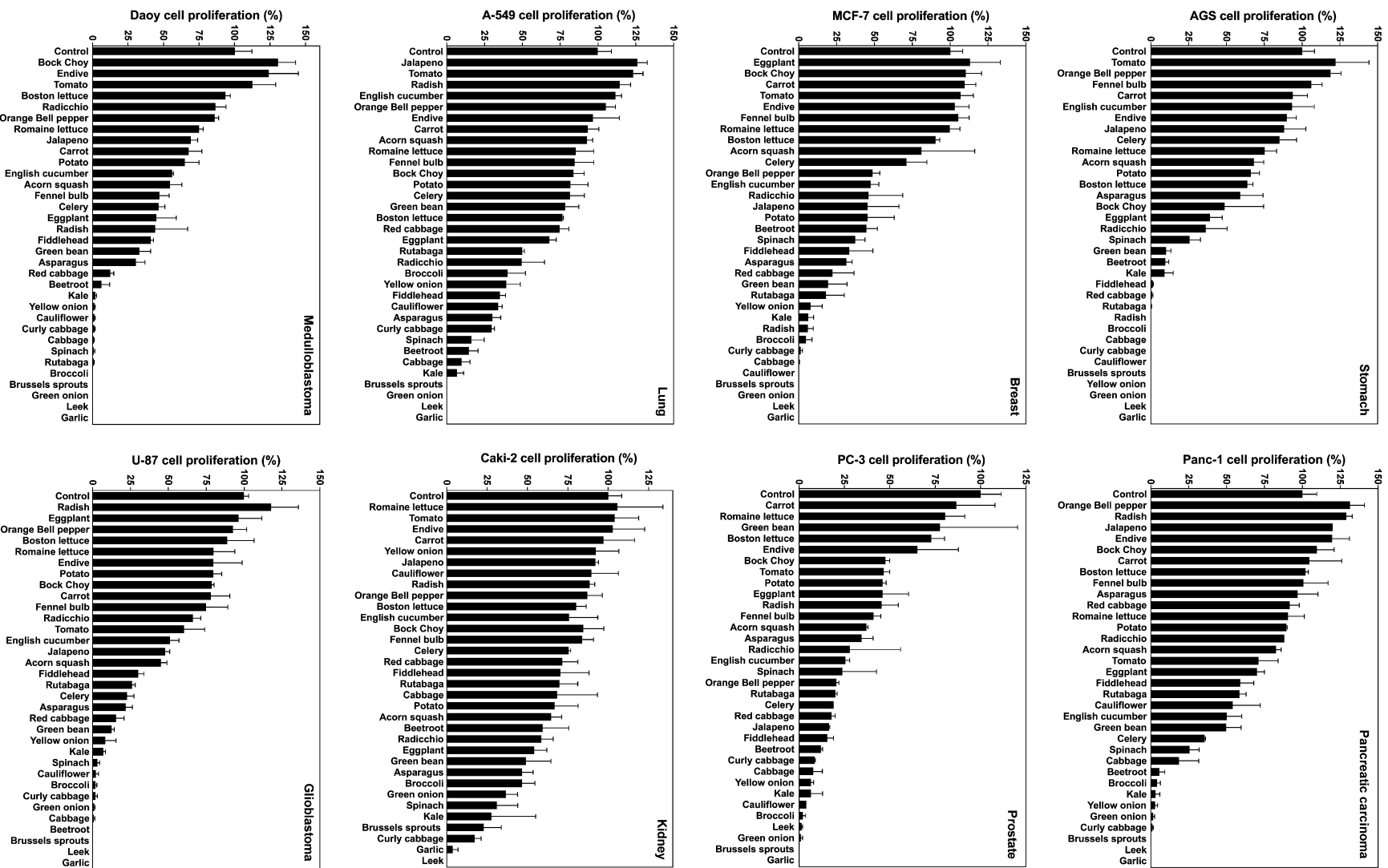


Fig. 1. Inhibition of tumour cell proliferation by vegetable extracts. Tumour cell lines derived from stomach adenocarcinoma (AGS), mammary gland adenocarcinoma (MCF-7), pancreatic carcinoma (Panc-1), prostatic adenocarcinoma (PC-3), lung carcinoma (A549), medulloblastoma (Daoy), renal carcinoma (Caki-2), and glioblastoma (U-87 MG) were incubated for 48 h in the absence or in the presence of a 1/20 dilution (corresponding to 166 mg raw vegetable per ml) of the indicated vegetable extracts. Cell viability was determined by assaying the mitochondrial activity of treated cells using the WST-1 assay. Results are the means \pm SD of 4 experiments performed in triplicates.

Table 1
Inhibition of cancer cell proliferation by vegetable juices

Cancer cell line	Number of vegetable extracts (>50% inhibition)
PC-3 (prostate)	23/34
AGS (stomach)	19/34
U-87 (glioblastoma)	18/34
DAOY (medulloblastoma)	17/34
MCF-7 (breast)	17/34
A-549 (lung)	14/34
Panc-1 (pancreas)	12/34
Caki-2 (kidney)	7/34

Table 2
Identification of vegetables with chemopreventive potential

Little	Intermediate	High	Very high
<i>Inhibitory effect on cancer cell proliferation^a</i>			
Acorn squash	Celery	Asparagus	Brussel sprouts
Bock choy	Eggplant	Beetroot	Cabbage
Boston lettuce		Broccoli	Curly cabbage
Carrot		Cauliflower	Garlic
Endive		Fiddlehead	Green onion
English cucumber		Green bean	Kale
Fennel bulb		Radish	Leek
Jalapeno		Red cabbage	Spinach
Orange sweet pepper		Rutabaga	
Potato		Yellow onion	
Radicchio			
Romaine lettuce			
Tomato			

^a Little activity is defined as an inhibitory effect of 50% on less than 2 cell lines. Intermediate activity is defined as an inhibitory effect of 50% on more than 2 but less than 4 cell lines. High activity is defined as an inhibitory effect of 50% on four and more cell lines. Very high activity is defined as an inhibitory effect of at least 50% on all cell lines.

inhibition at 1/100 dilution of the extract (33.2 mg raw vegetable/ml). The inhibitory effect of Brussels sprouts was slightly higher than that of green onion (60% at 1/100 dilution), while mature onion and broccoli inhibited proliferation by 30% at a 1/100 dilution, with complete inhibition being only observed at a 1/20 dilution (166 mg raw vegetable/ml).

In order to determine whether the strong inhibitory effects of cruciferous and *Allium* vegetables were specific for tumour cells, the effects of these extracts on the proliferation of normal fibroblast were subsequently monitored. As shown in Fig. 2b, all extracts had strong inhibitory activities against a glioblastoma cell line but had negligible effects on the growth of normal cells, strongly suggesting that the antiproliferative properties of these vegetables are specific to cells of tumour origin.

3.2. Antiproliferative and antioxidant activities

Oxidative stress is now recognized as a major factor associated with the development of chronic diseases, including cancer and cardiovascular disease (Ames et al., 1995). This has led to the hypothesis that the beneficial effects of fruits and vegetables could be largely explained by their high content of antioxidants (Prior, 2003). Antioxidant activity is involved in cancer prevention at the initiation stage while antiproliferative activity is targeting cancer cells at the promotion and progression stages (Manson, 2003; Surh, 2003). We measured the oxygen radical absorbance capacity (ORAC) of the extracts (Table 3), and found that there were significant differences between ORAC values of various vegetables. Garlic, curly cabbage and Brussels sprouts were the strongest source of antioxidants (41.1, 40.5, and 32.9 μmol Trolox equiv./ml, respectively) whereas other vegetables such as lettuce and cucumber contained considerably less antioxidants (1.5 and 1.4 μmol Trolox

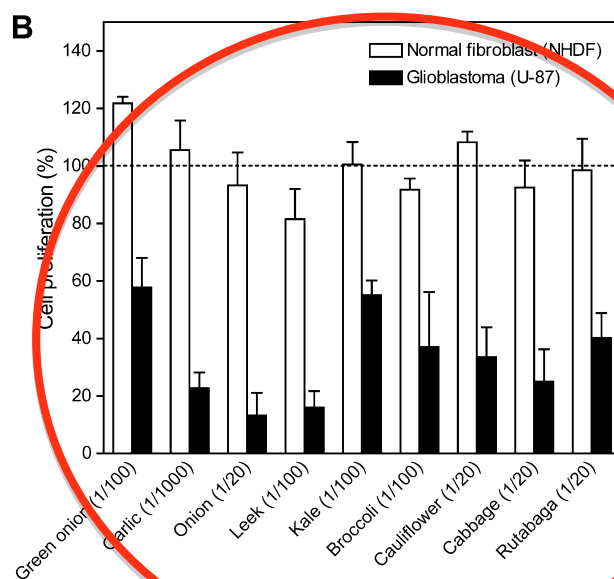
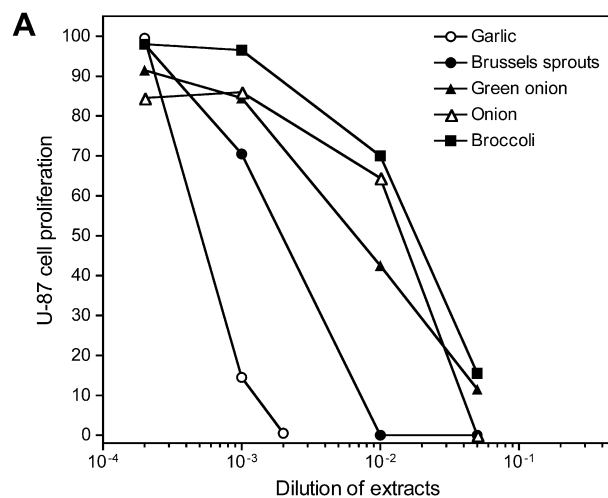


Fig. 2. Specificity of the antiproliferative activities of vegetable extracts against tumour cells. (a) Glioblastoma cells were incubated for 48 h with 1/20, 1/100, 1/500, 1/1000 and 1/5000 dilutions (corresponding to 166, 33.2, 6.64, 3.32 and 0.66 mg raw vegetable per ml, respectively) of the indicated vegetable extracts and the extent of proliferation was monitored as described in the legend to Fig. 1. (b) Glioblastoma cells (dark columns) or NHDF normal fibroblasts (open columns) were incubated with the indicated extracts and proliferation was determined. A representative experiment is shown.

equiv./ml). Our results suggest that both antioxidant and antiproliferative activities, involved in two different mechanisms of chemoprevention, could be considered for a better evaluation of the global anticancer potential of fruits and vegetables. The results are in agreement with reports showing that many powerful anticancer vegetables, including cruciferous vegetables such as broccoli and cauliflower, show modest antioxidant activities *in vitro* (Wu et al., 2004).

4. Discussion

Over 250 epidemiological studies have suggested that individuals consuming diets high in fruits and vegetables have a reduced risk of developing several cancers (World Cancer Research Fund & American Institute for Cancer Research, 1997). These observations form the basis of current recommendations from governmental health agencies around the world promoting consumption of at least 5 servings of these foods daily as a mean to reduce the inci-

Table 3
Antioxidant capacity, antiproliferative activity and protein concentration of vegetable juices

Vegetable extracts	Antioxidant capacity ($\mu\text{mol Trolox equiv./mL}$)	Antiproliferative activity (% inhibition)	Protein concentration (mg/mL)
Garlic	41.1 \pm 1.9	100	9.15 \pm 0.33
Curly cabbage	40.5 \pm 2.7	95 \pm 5	1.75 \pm 0.07
Brussels sprouts	32.9 \pm 0.5	100	4.94 \pm 0.23
Beetroot	24.7 \pm 1.8	100	1.54 \pm 0.05
Red cabbage	23.2 \pm 0.4	94 \pm 2	1.10 \pm 0.02
Fiddlehead	19.4 \pm 0.7	64 \pm 6	0.76 \pm 0.01
Spinach	17.4 \pm 0.5	99	5.45 \pm 0.27
Eggplant	15.5 \pm 2.0	1 \pm 6	0.95 \pm 0.04
Yellow onion	11.5 \pm 0.1	99 \pm 2	0.45 \pm 0.03
Green onion	10.6 \pm 0.5	100	1.02 \pm 0.01
Kale	9.7 \pm 1.2	100	4.32 \pm 0.10
Asparagus	9.2 \pm 1.2	71 \pm 1	2.16 \pm 0.02
Orange bell pepper	7.9 \pm 0.3	0 \pm 1	0.24 \pm 0.01
Potato	6.8 \pm 0.2	25 \pm 4	7.08 \pm 0.22
Broccoli	6.8 \pm 0.9	100	3.71 \pm 0.02
Radicchio	6.4 \pm 0.1	41 \pm 8	0.92 \pm 0.05
Radish	5.9 \pm 0.1	4 \pm 9	0.75 \pm 0.01
Leek	5.8 \pm 0.04	100	3.44 \pm 0.08
Rutabaga	5.1 \pm 0.3	71 \pm 1	0.75 \pm 0.02
Cauliflower	4.8 \pm 0.4	100	1.66 \pm 0.15
Cabbage	4.7 \pm 0.3	100	1.27 \pm 0.05
Jalapeno	4.7 \pm 0.2	49 \pm 4	1.13 \pm 0.03
Green beans	3.9 \pm 0.2	88 \pm 3	3.70 \pm 0.03
Romaine lettuce	2.9 \pm 0.2	0 \pm 1	1.71 \pm 0.05
Fennel bulb	2.7 \pm 0.4	2 \pm 10	0.79 \pm 0.01
Endive	2.5 \pm 0.1	0 \pm 1	0.44 \pm 0.01
Bock choy	2.1 \pm 0.5	19 \pm 1	1.02 \pm 0.03
Carrot	1.9 \pm 0.4	1 \pm 6	1.30 \pm 0.02
Celery	1.8 \pm 0.2	70 \pm 2	0.44 \pm 0.02
Tomato	1.6 \pm 0.1	16 \pm 1	0.07 \pm 0.01
Boston lettuce	1.5 \pm 0.3	0 \pm 12	0.99 \pm 0.03
Acorn squash	1.5 \pm 0.3	53 \pm 10	0.74 \pm 0.02
English Cucumber	1.4 \pm 0.2	41 \pm 11	0.27 \pm 0.02

dence of chronic diseases and cancer (Heimendinger & Chapelsky, 1996). However, fruits and vegetables contain varying levels of chemopreventive phytochemicals so that a global protective role of these foods is unlikely (McCullough & Giovannucci, 2004) and that increased consumption of certain foods with the highest phytochemical content must also be strongly encouraged (Johnston, Taylor, & Hampl, 2000). In this respect, in a large prospective study, total fruit and vegetable consumption was not associated with a reduction of the overall cancer incidence (Hung et al., 2004). Interestingly, a strong reduction of bladder cancer associated with the consumption of cruciferous vegetables was observed within this same male cohort (Michaud et al., 1999), again suggesting that specific foods or food groups have benefits against specific cancers. This is supported by numerous studies showing that consumption of cruciferous vegetables (Taalay & Fahey, 2001; Verhoeven, Goldbohm, van Poppel, Verhagen, & van den Brandt, 1996), *Allium* vegetables (Fleischauer & Arab, 2001; Galeone et al., 2006; Milner, 2001) or citrus fruits (Crowell, 1999) is consistently associated with strong protective effects against specific cancers. These results thus strongly suggest that the chemopreventive effects of fruits and vegetables should not be interpreted only in terms of the quantity of fruits and vegetables consumed by individuals but also must take into account the intake of particular foods or food groups with high anticancer properties.

There is increasing evidence that the chemopreventive properties of fruits and vegetables result from the additive and synergistic

effects of several phytochemicals present in these foods (Lee et al., 2004; Liu, 2003). In this respect, the use of vegetable extracts provide an interesting approach to assess the anticancer properties of a given vegetable since these extracts contain several bioactive molecules and are also more representative of the normal consumption of these food sources by humans (Brandi et al., 2004; Smith, Lund, Clarke, Bennett, & Johnson, 2005; Smith, Mithen, & Johnson, 2003). The vegetable juices used in this study contain a wide variety of phytochemicals but do not contain water-insoluble molecules such as lycopene found in tomatoes or β -carotene found in yellow, orange, and green leafy fruits and vegetables, including carrot, spinach, and broccoli. It is thus possible that our results underestimate the whole antioxidant and antiproliferative activities of specific vegetables containing water-insoluble bioactive phytochemicals.

The majority of the vegetable extracts tested in this study, including vegetables that are commonly consumed in Western countries such as potato, carrot, lettuce and tomato, had little effect on the proliferation of the tumour cell lines. The breast and prostate cancer cell lines were the only cancer cells showing some sensitivity to the potato extract, with a 50% inhibition of proliferation, while lettuce and carrot extracts had no significant effects on the proliferation of all the tested cell lines. The tomato extract was also ineffective in blocking tumour cell proliferation with the notable exception of the PC-3 prostate cell line, in agreement with the known chemopreventive effect of tomatoes on prostate cancer (Giovannucci, 2005).

We observed that cruciferous vegetables had potent inhibitory activities against most cancer cell lines. This inhibitory effect is most likely related to the content of these vegetables in glucosinolates (Fenwick, Heaney, & Mullin, 1983) since upon mechanical disruption of the vegetables, glucosinolates are rapidly converted to isothiocyanates, a highly reactive class of phytochemicals that potently inhibit several key events involved tumour cell growth (Keum, Jeong, & Kong, 2004; Thornalley, 2002). Brussel sprouts, which have the highest content in glucosinolates (McNaughton & Marks, 2005) were the most active cruciferous vegetable, with complete inhibition of the proliferation of all tumour cell lines, and was also one of few tested vegetable extract that strongly inhibited the growth of the kidney tumour cell line (Caki-). Other members of the *Brassica oleracea* species, such as kale, cabbage, curly cabbage, cauliflower and broccoli were also among the most inhibitory vegetables tested. Other cruciferous vegetables such as rutabaga, radicchio, radish and red cabbage were also inhibitory, although to a lesser extent, while Bock Choy, a Chinese cabbage that contains much less glucosinolates (McNaughton & Marks, 2003) showed moderate activity towards three cell lines (prostate, breast and stomach) but was inactive in all other cases.

We show in this work that vegetables that are the most commonly consumed in Western countries, such as potato, carrot, tomato and lettuce, had in general a weak effect on tumour cell proliferation. The lack of inhibitory effect of these widely consumed vegetables is noteworthy since potatoes, carrots, tomatoes and leaf lettuces account for approximately 60% of total per capita vegetable intake in the United States adult population. Potatoes, in particular, represent as much as 32% of vegetable consumption, half of this intake being in the form of French fries (Johnston et al., 2000; Krebs-Smith & Kantor, 2001). By contrast, the intake of dark green and cruciferous vegetables represent less than 1% of mean fruit and vegetable consumption and that of garlic is even lower (Krebs-Smith & Kantor, 2001). Thus, although the consumption of fruits and vegetables in general must still be strongly encouraged, specific recommendations regarding the need to eat a wide variety of these foods, including cruciferous, dark green and *Allium* vegetables, are clearly required in order

to increase the benefits of fruit and vegetable intake on cancer prevention.

The importance of eating a wide variety of vegetables is also well illustrated by the differential sensitivity of tumour cells to these foods. Thus, the antiproliferative effect of vegetable extracts markedly vary depending on the origin of the tumour and that no vegetable, with very few exceptions that will be discussed below, can be considered as effective against all types of cancer cells. Tumour cells derived from the kidney and the pancreas, two malignancies that are highly resistant to most chemotherapeutic regimens, were the least sensitive cancer cell lines tested in our study, with only 7 and 12 extracts showing an inhibitory activity greater than 50% on kidney and pancreatic cells, respectively. For example, a cauliflower extract, which markedly inhibit the proliferation of all other tested cell lines was much less active on Panc-1 cells and showed no significant inhibitory effect on Caki-2 cells. Dramatic differences in the inhibitory activity of some extracts were also observed, depending on the origin of the tumour cells. A good example is the effect of the radish extract, which completely abolished the proliferation of stomach and breast cancer cells but had no inhibitory effect on tumour cells of lung, pancreas, brain and kidney origin. On the contrary, the orange bell pepper extract, which was inactive against most tumour cell types, showed significant inhibitory activity (75% inhibition) against tumour cells of prostate origin. The large variation existing in the nature and in the levels of anticancer phytochemicals in vegetables and the differential sensitivity of tumour cells to these molecules thus imply that a diversified diet, containing several distinct classes of vegetables (and hence of phytochemicals) is essential for effective prevention of cancer.

This study also identified a number of cruciferous and *Allium* vegetables as foods with exceptional inhibitory activity against all tested cell lines, including those from the kidney and pancreas. Garlic, leek, immature (green) onion as well as a number of cruciferous vegetables, notably Brussels sprouts, kale, broccoli and various cabbages were found to possess very potent inhibitory activities against all tested cell lines. These properties are in agreement with the known anticancer properties of these vegetables observed in both epidemiological and laboratory studies (Fleischauer & Arab, 2001; Galeone et al., 2006; Milner, 2001; Talalay & Fahey, 2001; Verhoeven et al., 1996). These chemopreventive effects are likely related to the formation of organosulfur compounds following mechanical disruption of these vegetables, namely isothiocyanates from cruciferous and a series of allyl sulfur molecules from *Allium* vegetables (Fenwick et al., 1983; Pinto, Lapsia, Shah, Santiago, & Kim, 2001; Wu, Kassie, & Mersch-Sundermann, 2005).

The comparative assessment of the inhibitory activity of common vegetables strongly suggest that not all of these vegetables are equally protective against cancer. In spite of intensive efforts to sensitize Western populations to the benefits of fruit and vegetable consumption, the daily intake of these foods still remain very low and the overall spectrum of consumed fruits and vegetables is rather limited (Krebs-Smith & Kantor, 2001). It is especially noteworthy that vegetables that contain the highest anticancer properties, such as cruciferous, dark green and *Allium* vegetables, make up a miniscule amount of the overall fruit and vegetable consumption. Since the formation of tumours is a random event that occurs in a significant percentage of the adult population (Black & Welch, 1993), the increased consumption of these vegetables with high anticancer properties could play a central role in preventing these tumours to reach a clinical stage (Folkman & Kalluri, 2004) and thus reduce the incidence of several types of cancers.

Acknowledgements

This work was supported by Grant 10019 from the Cancer Research Society to R.B. We thank David Labbé for helpful suggestions.

References

- Ames, B. N., Gold, L. S., & Willett, W. C. (1995). The causes and prevention of cancer. *Proceedings of the National Academy of Sciences of USA*, 92, 5265–5268.
- Black, W. C., & Welch, H. G. (1993). Advances in diagnostic imaging and overestimations of disease prevalence and the benefits of therapy. *The New England Journal of Medicine*, 328, 1237–1243.
- Brandi, G., Schiavano, G. F., Zaffarano, N., De Marco, C., Paiardini, M., Cervasi, B., et al. (2004). Mechanisms of action and antiproliferative properties of *Brassica oleracea* juice in human breast cancer cell lines. *Journal of Nutrition*, 135, 1503–1509.
- Crowell, P. L. (1999). Prevention and therapy of cancer by dietary monoterpenes. *Journal of Nutrition*, 129, 775S–778S.
- Dávalos, A., Gómez-Cordovés, C., & Bartolomé, B. (2004). Extending applicability of the oxygen radical absorbance capacity (ORAC-Fluorescein) assay. *Journal of Agricultural and Food Chemistry*, 52, 48–54.
- Doll, R., & Peto, R. (1981). The causes of cancer: Quantitative estimates of avoidable risks of cancer in the United States today. *Journal of the National Cancer Institute*, 66, 1191–1308.
- Dorai, T., & Aggarwal, B. B. (2004). Role of chemopreventive agents in cancer therapy. *Cancer Letter*, 215, 129–140.
- Fenwick, G. R., Heaney, R. K., & Mullin, W. J. (1983). Glucosinolates and their breakdown products in food and food plants. *CRC Critical Reviews in Food Science and Nutrition*, 18, 123–201.
- Fleischauer, A. T., & Arab, L. (2001). Garlic and cancer: A critical review of the epidemiologic literature. *Journal of Nutrition*, 131, 1032S–1040S.
- Folkman, J., & Kalluri, R. (2004). Cancer without disease. *Nature*, 427, 787.
- Galeone, C., Pelucchi, C., Levi, F., Negri, E., Franceschi, S., Talamini, R., et al. (2006). Onion and garlic use and human cancer. *American Journal of Clinical Nutrition*, 84, 1027–1032.
- Gescher, A., Pastorino, U., Plummer, S. M., & Manson, M. M. (1998). Suppression of tumour development by substances derived from the diet – mechanisms and clinical implications. *British Journal of Clinical Pharmacology*, 45, 1–12.
- Giovanucci, E. (2005). Tomato products, lycopene, and prostate cancer: A review of the epidemiological literature. *Journal of Nutrition*, 135, 2030S–2031S.
- Heimendinger, J., & Chapelsky, D. (1996). The National 5 a day for better health program. *Advances in Experimental Medicine and Biology*, 401, 199–206.
- Hung, H.-C., Josphipura, K. J., Jiang, R., Hu, F. B., Hunter, D., Smith-Warner, S., et al. (2004). Fruit and vegetable intake and risk of major chronic disease. *Journal of the National Cancer Institute*, 96, 1577–1584.
- Keum, Y.-S., Jeong, W.-S., & Kong, A. N. T. (2004). Chemoprevention by isothiocyanates and their underlying molecular signaling mechanisms. *Mutation Research*, 555, 191–202.
- Johnston, C. S., Taylor, C. A., & Hampl, J. S. (2000). More Americans are eating “5 a day” but intakes of dark green and cruciferous vegetables remain low. *Journal of Nutrition*, 130, 3063–3067.
- Krebs-Smith, S. M., & Kantor, L. S. (2001). Choose a variety of fruits and vegetables daily: Understanding the complexities. *Journal of Nutrition*, 131, 487S–501S.
- Lamy, S., Gingras, D., & Béliveau, R. (2002). Green tea catechins inhibit vascular endothelial growth factor receptor phosphorylation. *Cancer Research*, 62, 381–385.
- Lee, K. W., Lee, H. J., & Lee, C. Y. (2004). Vitamins, phytochemicals, diets and their implementation in cancer chemoprevention. *CRC Critical Reviews in Food Science & Nutrition*, 44, 437–452.
- Liu, R. H. (2003). Health benefits of fruits and vegetables are from additive and synergistic combinations of phytochemicals. *American Journal of Clinical Nutrition*, 78, 517S–520S.
- Manson, M. M. (2003). Cancer prevention – the potential for diet to modulate molecular signalling. *Trends Molecular Medicine*, 9, 11–18.
- Martin, K. R. (2006). Targeting apoptosis with dietary bioactive agents. *Experimental Biology and Medicine*, 231, 117–129.
- McCullough, M. L., & Giovannucci, E. L. (2004). Diet and cancer prevention. *Oncogene*, 23, 6349–6364.
- McNaughton, S. A., & Marks, G. C. (2003). Development of a food composition database for the estimation of dietary intakes of glucosinolates, the biologically active constituents of cruciferous vegetables. *British Journal of Nutrition*, 90, 687–697.
- Michaud, D. S., Spiegelman, D., Clinton, S. K., Rimm, E. B., Willett, W. C., & Giovannucci, E. L. (1999). Fruit and vegetable intake and incidence of bladder cancer in a male prospective cohort. *Journal of the National Cancer Institute*, 91, 605–613.
- Milner, J. A. (2001). Mechanisms by which garlic and allyl sulfur compounds suppress carcinogen bioactivation. Garlic and carcinogenesis. *Advances in Experimental Medicine and Biology*, 492, 69–81.
- Naczki, M., & Shahidi, F. (2004). Extraction and analysis of phenolics in food. *Journal of Chromatography A*, 1054, 95–111.

- Naczek, M., & Shahidi, F. (2006). Phenolics in cereals, fruits and vegetables: Occurrence, extraction and analysis. *Journal of Pharmaceutical and Biomedical Analysis*, 41, 1523–1542.
- Pinto, J. T., Lapsia, S., Shah, A., Santiago, H., & Kim, G. (2001). Antiproliferative effects of garlic-derived and other *Allium* compounds. *Advances in Experimental Medicine and Biology*, 492, 83–106.
- Prior, R. L. (2003). Fruits and vegetables in the prevention of cellular oxidative damage. *American Journal of Clinical Nutrition*, 78, 570S–578S.
- Smith, T. K., Mithen, R., & Johnson, I. T. (2003). Effects of *Brassica* vegetable juice on the induction of apoptosis and aberrant crypt foci in rat mucosal crypts *in vivo*. *Carcinogenesis*, 24, 491–495.
- Smith, T. K., Lund, E. K., Clarke, R. G., Bennett, R. N., & Johnson, I. T. (2005). Effects of Brussels sprout juice on the cell cycle and adhesion of human colorectal carcinoma cells (HT29) *in vitro*. *Journal of Agricultural and Food Chemistry*, 53, 3895–3901.
- Surh, Y.-J., Chun, K.-S., Cha, H.-H., Han, S. S., Keum, Y.-S., Park, K.-K., et al. (2001). Molecular mechanisms underlying chemopreventive activities of anti-inflammatory phytochemicals: Down-regulation of COX-2 and iNOS through suppression of NF- κ B activation. *Mutation Research*, 480–481, 243–268.
- Surh, Y.-J. (2003). Cancer chemoprevention with dietary phytochemicals. *Nature Reviews Cancer*, 3, 768–780.
- Talalay, P. (2000). Chemoprotection against cancer by induction of phase 2 enzymes. *Biofactors*, 12, 5–11.
- Talalay, P., & Fahey, J. W. (2001). Phytochemicals from cruciferous plants protect against cancer by modulating carcinogen metabolism. *Journal of Nutrition*, 131, 3027S–3033S.
- Thornalley, P. J. (2002). Isothiocyanates: Mechanisms of cancer chemopreventive action. *Anticancer Drugs*, 13, 331–338.
- Tosetti, F., Ferrari, N., De Flora, S., & Albini, A. (2002). “Angioprevention”: Angiogenesis is a common and key target for cancer chemopreventive agents. *FASEB Journal*, 16, 2–14.
- Verhoeven, D. T. H., Goldbohm, R. A., van Poppel, G., Verhagen, H., & van den Brandt, P. A. (1996). Epidemiological studies on *Brassica* vegetables and cancer risk. *Cancer Epidemiology Biomarkers & Prevention*, 5, 733–748.
- Willett, W. C. (2002). Diet and cancer. *The Oncologist*, 5, 393–404.
- World Cancer Research Fund & American Institute for Cancer Research (1997). Food, nutrition, and the prevention of cancer: A global perspective. Washington, DC: American Institute for Cancer Research.
- Wu, X., Kassie, F., & Mersch-Sundermann, V. (2005). Induction of apoptosis in tumor cells by naturally occurring sulfur-containing compounds. *Mutation Research*, 589, 81–102.
- Wu, X., Beecher, G. R., Holden, J. M., Haytowitz, D. B., Gebhardt, S. E., & Prior, R. L. (2004). Lipophilic and hydrophilic antioxidant capacities of common foods in the United States. *Journal of Agricultural and Food Chemistry*, 52, 4026–4037.