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SSDI: 0308-8146(95)00256-1

Food Chemistry, Vol. 60, No. 2, pp. 161–164, 1997

0308-8146/97 $17.00+0.00

Influence of fruit and vegetable extracts on lipid peroxidation in microsomes containing specific cytochrome P450s

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(Accepted 1 November 1995)

We have examined the effect of 12 food extracts on iron/ascorbate-induced lipid peroxidation using microsomes enriched with specific cytochrome P450 isoenzymes, namely 1A1 and 3A4, prepared from human lymphoblastic cells. As a comparison, we also studied control microsomes, which contained negligible amounts of cytochrome P450. We observed antioxidant effects with both control and P450-containing microsomes in the case of grapefruit, green tea, coffee, tarragon and rosemary extracts. Pro-oxidant effects were observed for the brassica extracts (cabbage, cauliflower and Brussels sprouts) for all three microsome groups. Differences in the degree of lipid peroxidation between microsomes containing P450s 1A1/3A4 and control microsomes were seen for apple, tomato and parsnip peel extracts. The antioxidant properties of some of the food extracts were therefore modified by the presence of specific P450s. The results demonstrate the potential of P450-enriched microsomes in determining the antioxidant properties of food extracts and components. © 1997 Elsevier Science Ltd

INTRODUCTION.

Many dietary components have been shown to affect the biotransformation of xenobiotics, and may influence the toxicity and carcinogenicity of environmental chemicals (Schuster, 1989). Such compounds as flavonoids, phenolic acids, glucosinolates, sulphur compounds and indoles have been shown to alter the levels of phase I and phase II drug metabolising enzymes (Nebert & Negishi, 1982; Spencer *et al.*, 1991; Tephyly & Burchell, 1990). The most extensively studied phase I enzyme system is cytochrome P450, which comprises a multigene family of enzymes that plays a central role in the metabolism and disposition of a wide range of drugs, chemical carcinogens and endogenous compounds (Guengrich, 1988).

Understanding the exact role of cytochromes P450 and how biomolecules interact with them is complex because of their diverse functions (Bast & Haenen, 1984). Reconstitution experiments have been employed to yield information on substrate specificity and peroxidisability of the P450 isoenzymes (Ekstrom & Ingelman-Sundberg, 1989, Ohmori *et al.*, 1993). However, these studies require lengthy procedures of separation, purification, reconstitution and characterisation. To overcome these difficulties we have used microsomes from a panel of human lymphoblastoid cells, engineered to express individual cDNAs for specific human cytochrome P450s (Crespi *et al.*, 1993). These microsomes contain reasonable amounts of P450 isoenzymes, P450 reductase and cytochrome b_5 which compare favourably to human liver cells and are higher than in most reconstitution experiments (Ohmori *et al.*, 1993, Morehouse & Aust, 1988).

We have chosen three classes of microsomes: control microsomes, containing negligible amounts of P450; 1A1 microsomes; 3A4 microsomes. Isoenzyme cytochrome P450 1A1 is involved in polycyclic hydrocarbon metabolism and can be induced by certain foreign chemicals such as benzo(*a*)pyrene, 3-methylcholanthrene and β-naphthoflavone (Ohmori *et al.*, 1993). Cytochrome P450 3A4 is involved in the metabolism of steroid hormones, the bioactivation of aflatoxin B1 and the oxidation of nifedipine (Brian *et al.*, 1990). This P450 isoenzyme can be induced by dexamethane and rifampicin. In *in vitro* experiments, these P450s increased the amount of oxidative damage in microsomes subjected to ascorbate/iron-induced peroxidation (Lambert *et al.*, 1996). Peroxidation can be initiated by, for example, oxidation of Fe(II) with hydrogen peroxide, or the reduction of Fe(III) by ascorbate. We have shown that the cell microsomes can support iron/ascorbate-induced lipid peroxidation, but do not support NADPH-induced peroxidation. Furthermore, in our previous

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study we demonstrated that P450 does have an important role to play in non-enzymic microsomal lipid peroxidation, and an increase in peroxidative damage concomitant with an increase in cytochrome P450 was seen. In this communication we report our preliminary study into the identification of direct effects of food components on peroxidation as affected by specific cytochrome P450s.

MATERIALS AND METHODS

Materials

β-Lymphoblastoid cell microsomes were obtained from Gentest (Woburn, USA). Microsomes containing the 1A1 isozyme had 25 pmol P450 activity mg^{-1}, and microsomes containing 3A4 isoenzyme had 30 pmol mg^{-1} activity (values supplied by Gentest). Apples (cultivar Cox) and pears (cultivar Red Bartlett) were obtained from Norfolk Growers, UK. Authenticated cultivars of cruciferous vegetables—Brussels sprouts (cultivar Roger), cabbage (cultivar Savoy Rhapsody) and cauliflower (cultivar Diana)—were supplied by the National Institute of Agricultural Botany, UK. Rosemary, tarragon, green tea and coffee were obtained in powder form from local outlets. All biochemicals were of Analar grade (BDH, Poole, UK).

Methods

Preparation of fruit and vegetable extracts
A representative part of the fruit/vegetable that is normally eaten was weighed and rapidly frozen in liquid nitrogen, followed by freeze-drying and grinding to produce a fine powder. The process of chopping, pureeing or chewing involves some autolysis (i.e. hydrolysis of some of the plant compound by endogenous enzymes). To mimic this process, some samples, after drying and grinding, were moistened with water and incubated for 1 h at room temperature. Subsequently, 70% methanol (0.5 g per 30 ml) was added to the extracts and boiled for 15 min. Methanol was completely removed by rotary evaporation, and the sample volume adjusted with water. Extracts were centrifuged for 5 min at 5000*g*, and then sterile-filtered before use. Additionally, the Brussels sprouts extract was cooked by boiling for 15 min, and then extracted with methanol as described above.

Preparation of beverages and spices
The dried beverages and spices (0.5 g) were extracted in 30 ml of water (i.e. no methanol) and subsequently processed as detailed above.

Preparation of human cell microsomes
Cell microsomes (supplied in 1 ml aliquots, suspended in 100 mM potassium phosphate buffer, pH 7.0, at a protein concentration of 10 mg ml^{-1}) were washed to remove phosphate ions and cytosolic components which would interfere with subsequent assays (Gutteridge & Halliwell, 1990). Microsome suspensions (0.5 ml) were added to 150 mM KCl and centrifuged at 50000*g* for 10 min at 4°C. The resultant pellet was suspended in a total volume 0.5 ml of the same solvent, stored on ice and used within 8 h of preparation.

Assay of ascorbate/Fe(III)-induced peroxidation
Cell microsomes (0.4 mg ml^{-1}) were suspended in 150 mM KCl containing 0.5 mM $FeCl_3$ and 10 μl (330 μg) of the food extract. Peroxidation was initiated by the addition of ascorbate (final concentration 0.05 mM); the final volume was 0.4 ml. Samples were incubated at 37°C for 40 min followed by the termination of the reaction by the addition of 0.8 ml of 20% trichloroacetic acid, 0.4% thiobarbituric acid/0.25 M HCl and 10 μl of butylated hydroxytoluene (5%) in ethanol. The production of malondialdehyde was measured by the reaction with thiobarbituric acid at 80°C as described by Buege & Aust (1978).

Protein assay
Microsomal protein concentrations were determined by the bicinichoninic acid method (Smith *et al.*, 1985) using bovine serum albumin as the standard.

Table 1. Influence of the 12 food extracts on ascorbate/iron-induced microsomal lipid peroxidation

Extract	Lipid peroxidation (%) relative to value without addition of food extract		
	Control	1A1	3A4
Apple, A	81.6 ± 16.0	8.0 ± 4.0	68.2 ± 9.5
Pear, A	103.6 ± 12.5	97.2 ± 2.6	92.8 ± 19.9
Grapefruit, A	9.6 ± 5.7	6.6 ± 2.9	9.0 ± 4.6
Brussels sprouts, C	148.8 ± 10.2	128.2 ± 10.6	167.4 ± 7.9
Cabbage, A	138.4 ± 5.1	131.8 ± 4.4	140.6 ± 5.7
Cauliflower, A	147.3 ± 4.1	133.2 ± 6.7	155.6 ± 10.4
Tomato, A	110.6 ± 7.6	35.0 ± 12.0	6.1 ± 1.3
Parsnip peel, A	97.8 ± 3.7	96.2 ± 7.1	8.2 ± 3.2
Green tea, W	6.7 ± 3.1	3.2 ± 0.9	4.5 ± 0.9
Coffee, W	4.2 ± 1.5	3.0 ± 0.3	4.5 ± 9
Tarragon, W	6.5 ± 3.3	4.0 ± 2.8	4.3 ± 1.8
Rosemary, W	4.7 + 2.0	3.7 ± 0.9	4.5 ± 1.7

Extracts (330 μg dry weight) were processed as follows: A, autolysed extract; C, cooked extract; W, water extract. Amount of lipid peroxidation (absorbance at 535 nm) for each class of microsomes, without the addition of food extract: control microsomes, 0.60 ± 0.04; 1A1 microsomes, 0.72 ± 0.05; 3A4 microsomes, 0.72 ± 0.05. Experiments were performed at least five times.

RESULTS.

Table 1 summarises the influence of the 12 food extracts on ascorbate/iron-induced microsomal lipid peroxidation. The two beverages, tea and coffee, and the two spices, rosemary and tarragon, were all potent inhibitors of lipid peroxidation (3–7%) for all three classes of microsomes. Contrastingly, all three brassica extracts (cauliflower, cabbage and Brussels sprouts), were pro-oxidant in all three microsome groups and increased the level of peroxidation significantly (128–168%).

The three fruit extracts each demonstrated different effects in the assay. Grapefruit was a potent inhibitor of lipid peroxidation with all three microsomes (6–10%). Apple showed a differential effect, having a weak inhibitory effect for control (81%) and 3A4 microsomes (68%) and a strong inhibitory effect with 1A1 microsomes (8%). Pear demonstrated no effect.

Tomato and parsnip peel also produced a differential effect between the classes of microsomes. Parsnip peel was an excellent inhibitor of lipid peroxidation with 3A4 microsomes (8%), but had no effect with the other two microsomes (96–98%). Tomato showed a similar trend as parsnip peel except partial inhibition (35%) was seen with 1A1 microsomes.

The amount of lipid peroxidation of 1A1 and 3A4 microsomes without the addition of food extract was identical, while that of control microsomes was significantly lower, confirming a previous study where the degree of peroxidation was a function of the total amount of P450 present (Lambert *et al.*, 1996).

DISCUSSION

The availability of human lymphoblastoid cell microsomes, expressing specific P450s, has offered a practical alternative to reconstitution experiments for studying P450s and their role in lipid peroxidation in biological membranes. The levels of both cytochrome P450 and the reductase are both higher than those used in reconstitution experiments.

The pro-oxidant effect of the three brassica extracts clearly does not involve the cytochrome P450 system, since peroxidation of control microsomes and 1A1/3A4 microsomes led to the same level of membrane damage. This contrasts with our previous study where we reported on the antioxidant nature of brassica extracts (Plumb *et al.*, 1996). We found cooked Brussels sprouts to be pro-oxidant, but all the other brassicas (cabbage, cauliflower, etc.) showed antioxidant properties. Although there have been many studies on the induction of antioxidant enzymes by compounds from brassicas, few studies have focused on their direct antioxidant and free-radical scavenging properties.

The antioxidant effects observed with grapefruit, green tea, coffee, tarragon and rosemary were not dependent on the presence of P450. This is consistent with previous results on human liver microsomes, since the extracts mentioned above all contain well-documented direct antioxidants: for examapale, carnosol in rosemary (Arouma *et al.*, 1992) and catechins in green tea (Scott *et al.*, 1993).

The results observed with apple, tomato and parsnip peel extracts are more intriguing. The effects must be due to the direct action of compounds (derived from the food extracts) on the P450s since the levels of lipid peroxidation in the control microsomes were unaffected. This is also further evidence that cytochrome P450 does have an influence on the so-called 'non-enzymic' lipid peroxidation. Work is ongoing, involving the three extracts mentioned above, to identify the bioactive molecule(s) responsible for our observations.

This study represents a preliminary investigation into the direct effects of dietary components on the role of cytochrome P450 in peroxidation. We identified three food extracts that demonstrated an effect on P450 in *in vitro* peroxidation. The availability of cell microsomes containing specific cytochrome P450s offers an opportunity to study in more detail the interaction of dietary components with cytochrome P450s and the resulting effect on peroxidation.

ACKNOWLEDGEMENTS

We would like to thank the Ministry of Agriculture, Fisheries and Food for funding this work.

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