JPP Journal of Pharmacy and Pharmacology

JPP 2006, 58: 1545–1552

Received January 4, 2006
Accepted July 24, 2006
DOI 10.1211/jpp.58.11.0016
ISSN 0022-3573

In-vitro mutagenic potential and effect on permeability of co-administered drugs across Caco-2 cell monolayers of *Rubus idaeus* and its fortified fractions

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Funding: This work was supported by the National Technology Agency of Finland via two projects during the years 2003–2005, and by Pro-Kinase Research LSHB-2004-503467 grant.

Abstract

This study investigated the mutagenic, anti-mutagenic and cytotoxic effects of acetone extract of raspberry, *Rubus idaeus* L. (v. Ottawa) Rosaceae, and the isolated and characterized ellagitannin and anthocyanin fractions thereof, suitable for food applications. The studied raspberry extract and fractions did not show any mutagenic effects determined in the miniaturized Ames test and were not cytotoxic to Caco-2 cells at the used concentrations. However, the anti-mutagenic properties were changed (i.e. decreased mutagenicity of 2-nitrofluorene in strain TA98, and slightly increased mutagenicity of 2-aminoanthracene in strain TA100) with metabolic activation. Further, their influence on the permeability of co-administered common drugs (ketoprofen, paracetamol, metoprolol and verapamil) across Caco-2 monolayers was evaluated. The apical-to-basolateral permeability of highly permeable verapamil was mostly affected (decreased) during co-administration of the raspberry extract or the ellagitannin fraction. Ketoprofen permeability was decreased by the ellagitannin fraction. Consumption of food rich in phytochemicals, as demonstrated here with chemically characterized raspberry extract and fractions, with well-absorbing drugs would seem to affect the permeability of some of these drugs depending on the components. Thus their effects on the absorption of drugs in-vivo cannot be excluded.

Introduction

A food can be regarded as functional if it is satisfactorily demonstrated to affect beneficially one or more target functions in the body, beyond adequate nutritional effects, in a way that is relevant to either an improved state of health and well-being or reduction of risk of disease (Diplock et al 1999). Because interest in preventive health care has increased, the food industry is producing not only added value products with minerals and vitamins, but also functional foods with various ingredients. These could be fractions of extracts of herbs, berries and other plant materials proven to have beneficial activity, such as antioxidative and antimicrobial (Tsao & Humayoun Akhtar 2005). Such enrichment could, however, result in increased toxicity or mutagenicity as well as interactions if drugs were taken contemporaneously. Some drugs appear to be safe when taken with food but less so if taken with fortified foods (Rates 2001; Wallace & Amsden 2002). Mechanisms of interactions between drugs and fortified foods are similar to well-known mechanisms of interactions between drugs and minerals (chelation, adsorption) and between drugs and secondary metabolites. For example, a secondary metabolite of the flavonoid naringenin that is present in grape juice is a known cytochrome P 450 enzyme inhibitor. It has also been found that eating raspberries at the same time with acetylsalicylic acid or ketoprofen can lead to haemorrhage as a result of additive antiplatelet effects (Abebe 2002; Wallace & Amsden 2002; Zhou et al 2005). Berries, which are traditionally a part of the Finnish diet, are good sources of phenolics with the predominating group of flavonoids, especially in red berries, being anthocyanins (Häkkinen et al 1999; Kähkönen et al 2001; Määttä-Riihinen et al 2005). Microbial glucosidases and glucuronidases in the colon can possibly affect the bioactivity of glycosylated compounds by deconjugating or otherwise modifying them (Scalbert et al 2002).

The bacterial reverse mutagenicity test on *Salmonella typhimurium* strains (Ames et al 1975a, b) is widely used as an initial screen to assess the mutagenic potential of substances (drugs) often required by regulatory authorities. This test is designed for the detection of the mutagenic potential of investigated substances through the induction of reverse mutations in the *His* gene of modified *S. typhimurium* strains. Agents produced by plants have been used as antimicrobial substances for a long time. Most of these antimicrobial compounds are secondary metabolites, including flavonoids, and produced as defence mechanism against microorganisms, insects and herbivores. Still only a small percentage of compounds produced by plants has been tested for detecting mutagens or anti-mutagens (Horn & Vargas 2003). Interestingly, some orange juices have been found mutagenic in the Ames *Salmonella* assay and the mutagenicity has been confirmed to be the result of phenolic compounds and vitamin C (Franke et al 2004). Compounds may also act like anti-mutagens by affecting other drugs or toxicants in many ways (i.e. changing the absorption rates and increasing uptake, reacting with the drug, competing with the drug for binding to plasma or by affecting activation and detoxification systems). Some plant extracts, tannins and their derivates, as well as flavonoids, have been reported to have anti-mutagenic activity (Edenharder et al 1993; Waters et al 1996; Horn & Vargas 2003).

Caco-2 cell cultures are widely used as an in-vitro model in drug-absorption studies. The model is useful in determining roles exhibited by various physical and biochemical barriers to drug absorption (Artursson 1990; Artursson et al 1996; Gan & Thakker 1997). Caco-2 cells have many properties similar to those of the enterocytes of the small intestine. They contain active transport and efflux proteins. According to the FDA, Caco-2 cell cultures can be used as an in-vitro model in bioavailability/bioequivalence testing of highly soluble drugs that permeate cell layers well (Amidon et al 1995; Artursson et al 1996), together with in-vitro dissolution tests. The capacity of a potential new drug, or natural compounds (Tammela et al 2004), to permeate absorptive cell layers can also be determined using Caco-2 cell cultures. An in-vitro absorption model of this kind has also been used to investigate whether the permeability of a drug is affected by other drugs (e.g. Laitinen et al 2003) or by food components (Laitinen et al 2004; Vuorela et al 2005) administered at the same time.

In this investigation a 6-well plate method for *S. typhimurium* strains TA98 and TA100, called a mini mutagenicity test (MMT), which is a validated miniaturized version of the regulatory Ames test protocol developed by Flamand et al (2001), was carried out. We also performed toxicity and permeability studies of the effects on co-administered drugs, in Caco-2 cell model with a natural product extract of red raspberry and its fortified fractions, which were aimed as components for functional foods, to assess their possible health-related risks.

Materials and Methods

General materials

Cell culture materials used for the mini mutagenicity and anti-mutagenicity tests and for Caco-2 assays are described in Vuorela et al (2005) and Laitinen et al (2004), respectively. Ketoprofen and verapamil hydrochloride were obtained from ICN Biomedicals Inc. (Aurora, OH) and metoprolol bitartrate from Sigma Chemical Co. (St Louis, MO). Paracetamol was donated by Orion Pharma (Espoo, Finland). All organic solvents were of HPLC grade.

Plant samples and characterization

Fresh red raspberries (*Rubus idaeus* L. (v. Ottawa) Rosaceae) (5 kg) were purchased from a market during the growing season of July–August 2003 in Finland. They were packed immediately into vacuum and stored at −18°C. The frozen raspberries were freeze-dried and extracted according to Kähkönen et al (2001). Interfering sugars were removed by solid phase extraction (C18) before analytical HPLC analysis for the determination of the phenolic profiles according to Kähkönen et al (2001). In short, the separation of phenolic compounds was carried out on a Nova-Pak C18 column (150 mm × 3.9 mm, 4 μm; Waters) with a WISP 712 autosampler, three 501 pumps, a PDA996 diode array detector and a Millennium 2020C/S software data module (Waters, Milford, MA). On the basis of spectral identification, phenolics were quantified in six subclasses: flavanols and proanthocyanidins (expressed as (+)-catechin equivalents; detection wavelength 280 nm), hydroxybenzoic acids (as gallic acid equivalents, 280 nm), ellagitannins (as ellagic acid equivalents, 280 nm), hydroxycinnamic acids (as chlorogenic acid equivalents, 320 nm), flavonols (as rutin equivalents, 365 nm) and anthocyanins (as cyanidin 3-glucoside equivalents, 520 nm). The standard compounds used were obtained from Extrasynthese (Genay, France) and Sigma Chemical Co. (St Louis, MO).

Raspberry ellagitannins and anthocyanins were isolated using the method described by Kähkönen et al (2003). The purity of the ellagitannin fraction was determined by HPLC at a wavelength of 280 nm. For identification of ellagitannin monomers, preparative HPLC followed by LC-ESI-MS analysis was used (Kähkönen et al 2003). The anthocyanin-containing fraction was further purified by using preparative HPLC followed by analytical HPLC for compositional determination (Kähkönen et al 2006). The samples were dissolved in HBSS for the assays.

Salmonella mutagenicity/anti-mutagenicity assay

The mutagenic activity of the samples was determined using the mini version of the incorporation assay described by Maron & Ames (1983) (i.e. the mini mutagenicity test (Flamand et al 2001)). In the assay, genotyped *Salmonella typhimurium* strains TA98 or TA100 (Xenometrix Inc., San Diego, CA) without or with 10% of Aroclor-induced rat liver S9 protein mix (according to Maron & Ames 1983) per well for metabolic activation were added to 6-well plates, incubated for 48 h at 37°C and colonies scored either manually or automatically by image analysis (GeneGenius Bio Imaging System; Syngene, Cambridge, UK) using GeneSnap acquisition software and GeneTools match analysis software. Possible bactericidal effect was evaluated according to Mortelmans & Zeiger

(2000). As positive controls, 0.1 μg/well of 2-nitrofluorene (2N; Sigma-Aldrich, Germany) or 0.05 μg/well of sodium azide (Na; Merck & Co, Germany) were used for TA98 and TA100, respectively. With metabolic activation, 2-aminoanthracene (2A; Sigma-Aldrich, Germany) was used as positive control (0.5 μg/well for TA98 and 0.75 μg/well for TA100). HBSS was used as the negative control (spontaneous revertant counts) and gave TA98 and TA100 values of 5–10 and 32–42 revertant colonies per well without metabolic activation and of 7–10 and 34–53 revertant colonies per well with metabolic activation, respectively. In the anti-mutagenicity assay based on Yen et al (2001) samples were added in wells with positive controls and colonies were scored as above. All determinations were performed in triplicate.

In-vitro Caco-2 permeation study

The Caco-2 cell line was obtained from the American Type Culture Collection (ATCC No. HTB-37; Rockville, MD) and cultured according to Laitinen et al (2004). Briefly, the cells were grown in a DMEM-medium containing 4.5 g L^{-1} glucose, supplemented with 10% fetal bovine serum, 1% non-essential amino acids, 1% L-glutamine, penicillin (100 IU mL^{-1}), and streptomycin (100 μg mL^{-1}). Cultures were maintained at 37°C in an atmosphere of 5% CO_2, 95% air and 95% relative humidity. All permeability experiments were performed under sink conditions (i.e. the quantity of transported compound in the acceptor chamber did not exceed 10% of the quantity in the donor compartment). The data were evaluated by comparing the samples at various time points with initial drug concentration reduced by the amount of previous samples. The permeability of the drug compounds (ketoprofen, paracetamol, metoprolol and verapamil) was studied in apical-to-basolateral (AP-BL; pH 7.40) direction at concentrations of 0.25 mM.

Cells from passage numbers 31–42 were used for the transport experiments at ages 21–28 days. Before the permeability experiments, the cell monolayers were washed twice with HBSS containing 10 mM HEPES, pH 7.40. Then cells were allowed to equilibrate in the transport buffer for 30 min. Transepithelial electrical resistance (TEER) was measured with a EVOMX voltohmmeter (EVOM; World Precision Instruments Inc., USA) before washing and after equilibration. The cell inserts with TEER values below 250 Ω cm^2 were discarded. Typically TEER values were 500–600 Ω cm^2 before experiments. The apical solution was changed to HBSS containing the drugs alone or with the raspberry samples (0.01, 0.1 and 1.0 mg mL^{-1}). After 15, 30, 60, and 90 min the cell inserts were moved into new wells with fresh HBSS. All experiments were conducted in triplicate. After the experiment, the cell cultures were washed once with HBSS and the TEER values were measured to be sure that monolayers were not damaged during the experiments. Mass balance was evaluated based on the sum of the cumulative amount transported to the acceptor compartment and remaining in the donor compartment against initial amount in the donor compartment. Recoveries (%, n = 9) of metoprolol, ketoprofen, verapamil and paracetamol were 97 ± 2, 95 ± 7, 79 ± 2 and 78 ± 2, respectively. The results indicate that none of the studied compounds were retained markedly in cellular structures or adsorbed to the plastic device or disintegrated during the experiment. The permeation samples were kept at −70°C until analysed.

The drug concentrations in the permeation experiments were analysed by HPLC (Waters Millennium, USA) with the Waters 486 Tunable Absorbance Detector, the Waters 717 plus Autosampler, and the Waters 510 pump according to Laitinen et al (2004). The analysis of metoprolol and verapamil was based on the method of Kunta et al (1997) and the analyses of ketoprofen and paracetamol were based on the method of Owen et al (1987) and Shim & Jung (1992), respectively. Linearity was assessed by performing measurements at several analyte concentrations. The quantitation ranges and retention times of metoprolol, verapamil, ketoprofen and paracetamol were 0.21–21.4 μg mL^{-1} (rt ~2.3 min), 0.05–20.16 μg mL^{-1} (rt ~4.1 min), 0.08–19.05 μg mL^{-1} (rt ~5.5 min) and 0.13–30.4 μg mL^{-1} (rt ~4.6 min), respectively, containing six points. Three quality control samples were analysed with every run duplicate. The values did not differ more than 10% from the expected value.

Cytotoxicity assay

MTT, a tetrazolium salt, was used for a colorimetric assay to determine viability of Caco-2 cells according to Mosmann (1983). After 90 min, test compound treatment absorbance data was collected using an ELISA plate reader at 550 nm. Assays were performed using 96-well plates, four replicates on the same plate.

Data analysis

Mutagenic or anti-mutagenic ratio (R) was calculated between the number of revertants at a given concentration of positive controls and the revertants in the corresponding samples. $R \geq 2$ was set as level for mutagenicity, whereas anti-mutagenicity was observed if the ratio was lower with sample than positive control alone.

The cumulative amount of the permeated compounds across Caco-2 cell monolayers was calculated from the concentrations measured in the receiver (basolateral) compartments. The apparent permeability coefficients, P_{app} ($cm\,s^{-1}$), were calculated according to the equation:

$$P_{app} = (\Delta Q/\Delta t)/(A \cdot C_0) \quad (1)$$

where $\Delta Q/\Delta t$ is the flux of the compound across the monolayers, A (cm^2) is the surface area of the cell monolayer, and C_0 ($\mu g\,mL^{-1}$) is the initial compound concentration in the donor (apical) compartment. Data are presented as the average P_{app} ($cm\,s^{-1}$) ± s.d. (n = 3). The P_{app} values of drug compounds with the studied raspberry samples were compared with those without extracts (controls), and the difference in the P_{app} was calculated as a percentage deviation of the P_{app} of the control. The results were confirmed statistically using unpaired *t*-test combined with Dunn–Sidak Adjusted Probability and Bonferroni Adjusted Probability

tests (SYSTAT version 11 for Windows; SYSTAT Software Inc., Richmond, CA). A significance level of 5% was used.

Results and Discussion

Phytochemical study of *Rubus idaeus* berry extract and fractions

The phenolic composition of the raspberry acetone extract and fractions used in this study is shown in Table 1. Polyphenols modulate the activity of a wide range of enzymes and cell receptors. In addition to having antioxidant properties, polyphenols have several other specific biological actions that are as yet poorly understood (Manach et al 2004; Vuorela et al 2004).

The freeze-dried extract in our studies contained about 50% of ellagitannins of the total phenolics (Table 1). Ellagitannins are esters of hexahydroxydiphenic acid and a polyol, usually glucose or quinic acid. Berries, especially of the family Rosaceae, genus *Rubus* (red raspberry, arctic bramble and cloudberry), are rich in ellagitannins (Clifford & Scalbert 2000; Kähkönen et al 2001; Mullen et al 2002). These berries produce only ellagitannins based on stable glucose conformation. In addition to pentagalloylglucose these berries contain dimeric or polymeric ellagitannins with only small amounts of monomers (Haslam 1989). All *Rubus* berries contain a dimeric form called sanquin H6 (Haddock et al 1982; Tanaka et al 1993). According to Tanaka et al (1993), raspberries contain tetrameric ellagitannin, lambertianin D. Ellagitannins from red raspberry have exhibited good radical scavenging activity in the DPPH assay and suppression of pro-inflammatory mediator PGE_2 (Vuorela et al 2005).

The raspberry used in our study contained anthocyanins, 10% of the total phenolics in the freeze-dried extract (Table 1). The flavonoids, which share a common structure consisting of two aromatic rings that are bound by three carbon atoms forming an oxygenated heterocycle, may themselves be divided into six subclasses as a function of the type of heterocycle involved: flavonols, flavones, isoflavones, flavanones, anthocyanidins and flavanols (catechins and proanthocyanidins). In addition to this diversity, polyphenols may be associated with various carbohydrates and organic acids and with one another. Foods rich in anthocyanins are aubergine, blackberry, black currant, blueberry, cherry, strawberry, red wine and black grape (Manach et al 2004). Raspberry anthocyanins have shown good antioxidant activity toward oxidation of liposomes (Kähkönen & Heinonen 2003) and, further, Viljanen et al (2004) found raspberry anthocyanins to be the most active berry phenolics in inhibiting the oxidation of a liposome–protein model system. According to Castelli et al (1999), liposomes are a suitable model for studying the membrane structure and properties due to their structural similarity to the lipid matrix of cell membranes.

Table 1 The composition of the main phenolics identified in raspberry extract and its fortified fractions

Sample	Phenolic composition (mg/g freeze-dried extract)	
Acetone extract	Total phenolics	554
	Ellagitannins	260
	Ellagic acid	24
	Anthocyanins	56
	Flavanols and proanthocyanidins	1.8
	Flavonols	1.2
	Hydroxybenzoic acids	0.8
	Hydroxycinnamic acids	(not detected)
Ellagitannin fraction	Ellagitannins[a]	356
	Ellagic acid	72
	Anthocyanins	4.5
	Flavonols	3
	Flavanols and proanthocyanidins	3
	Hydroxybenzoic acids	0.9
Anthocyanin fraction	Anthocyanins[b]	696
	Flavonols	2.4
	Hydroxycinnamic acids	7

[a]Mixture of monomeric (MW~ 936 g mol^{-1}), dimeric (including sanquin H6) and polymeric ellagitannins. [b]60% cyanidin-3-sophoroside, 16% cyanidin-3-glucoside, 15% cyanidin-3-glycosylrutinoside, 5% cyanidin-3-rutinoside, 2% pelargonidin-3-sophoroside, < 1% pelargonidin-3-glucoside, < 1% pelargonidin-3-rutinoside.

Mutagenicity, anti-mutagenicity and cytotoxicity assays

Botanical or herb-based food items may contain individual phytochemicals that are toxic (Schwarz & Roots 2003; Rietjens et al 2005: Zhou et al 2005). Neither the red raspberry extract nor its fortified fractions were cytotoxic to Caco-2 cells. Over the past ten years food manufacturers have become increasingly interested in polyphenols. The main reasons for this interest is the recognition of the antioxidant properties of polyphenols, their great abundance in our diet and their probable role in the prevention of various diseases associated with oxidative stress, such as cancer and cardiovascular and neurodegenerative diseases (e.g. Hertog et al 1993, 1997; Knekt et al 1996, 1997, 2000; Hollman & Katan 1999). Several phytotoxins cause further concern because of their bioactivation to reactive alkylating intermediates, causing cellular toxicity and genotoxicity resulting in tumours. The Ames test is a method for detecting carcinogens and mutagens with the *Salmonella* mutagenicity test, the method of which was first described by Ames et al (1975a, b). The miniaturized Ames test adapted in this study gave similar results for reference compounds (positive controls) as described in Flamand et al (2001), as well as for the Ames test according to Maron & Ames (1983). Positive control concentrations were selected so that the mutagenic ratios of them were over 2. None of the samples showed any mutagenicity, either with or without metabolic activation, on *S. typhimurium* strains TA98 and TA100. Many different *Salmonella* strains are used in the Ames test, but using the two strains TA98 and TA100 has been found good enough to detect most

mutagens (Kier et al 1986), although more extensive combinations of strains have been also implemented (e.g. Bonneau et al 1991). Also, no bactericidal effects were seen as lack of background growth caused by the spontaneously reversed bacteria in wells.

Anti-mutagenicity was evaluated against positive controls and R calculated. Comparing R values, anti-mutagenicity was observed if the ratio was lower with test sample than just positive control. The ratio between the number of revertants at a given concentration of positive controls and the revertants in the corresponding solvent control (HBSS) was calculated and the results are shown in Table 2. All the raspberry samples inhibited mutagenicity induced by 2-nitrofluorene against *S. typhimurium* strain TA98 without S9 mix, whereas with metabolic activation no inhibition occurred (i.e. the compounds changed into non-active). An increase of mutagenicity could be observed for all samples in the case of strain TA100 with S9 mix, but not without the metabolic activator. Pomegranate peel extracts that contain ellagic acid, ellagitannins and gallic acids have shown anti-mutagenicity against TA100 and TA1535 strains in studies made by Negi et al (2002). In our study, a slight decrease of anti-mutagenicity by the ellagitannin fraction could be detected for TA100 without metabolic activation. *Origanum* essential oil and carvacrol are other plant components that have been tested as antimutagens against *S. typhimurium* TA98 and TA100 with and without metabolic activator (S9), and both showed the ability to inhibit mutagenicity induced by positive controls (Ipek et al 2005).

Permeability studies in Caco-2 cells of co-administered drug compounds

In this study we evaluated the effects of raspberry extract and fractions on Caco-2 cell permeability of four commonly used drugs. The obtained P_{app} values from transport experiments with metoprolol, ketoprofen, verapamil and paracetamol were 32.5 ± 2.4, 27.4 ± 5.9, 27.8 ± 1.7 and 22.0 ± 1.9, respectively. The permeability of all studied compounds was high. All of the studied compounds are mainly transported via the transcellular route. The permeability of ketoprofen is assumed also to be mediated by monocarboxylic acid transporter MCT1 (Tamai et al 1995; Choi et al 2005). Verapamil is a well-known substrate of efflux protein P-glycoprotein (Tsuruo et al 1981; Orlowski et al 1996; Collet et al 2004). At the concentration 0.25 mM used, most probably the active efflux is saturated (Garrigos et al 1993; Balimane et al 2006) so that effect of P-gp activation could not bee seen. Only at very low donor concentrations is the effect of P-gp visible (Doppenschmitt et al 1999; Balimane et al 2006).

According to the partition theory, the relative contribution of the un-ionized form of acids is bigger in acid environments; meanwhile basic compound behave in the opposite way. The effects of medium pH on the permeability of Caco-2 monolayers largely depend on the dissociation condition in the solution and the permeability characteristic of each drug (Yamashita et al 2000). The relative contributions of the un-ionized form of basic metoprolol (pK_a 9.7) and verapamil (pK_a 8.7) are about 0.5% and 5% at pH 7.40, respectively, while at pH 7.0 corresponding values are 0.2% and 2%. The permeability of these basic drugs reduced to about 80% of the control value when pH changed from apical 7.4 to 7.0 (Laitinen et al 2004). On the contrary, the relative contribution of the un-ionized form of acidic ketoprofen (pK_a 5.9) is about 3.1% at pH 7.40, while 7.4% is un-ionized at pH 7.00. The permeability was 1.35 times greater at pH 7.00 than at pH 7.40. The permeability of very weakly acidic paracetamol (pK_a 9.5) should not be affected at these pH levels. The permeability of the compound studied could be under- or overestimated based on selected experiment conditions. The lumen situation is more complicated due to variable pH in different parts of the intestine. Since we cannot mimic the situation in the small intestine totally, we performed the experiments in traditionally used pH 7.4 both in the apical and the basolateral compartments. Figure 1 shows the results of the permeability studies of metoprolol, ketoprofen, verapamil and paracetamol contemporaneously administered with the different raspberry samples. All studied samples slightly decreased metoprolol permeability, though this effect was so minor that severe adverse effects are unlikely with this medicine and these raspberry samples. Permeability of verapamil decreased markedly during co-administration of the raspberry extract (ellagitannin content 50% of total phenolics) and the ellagitannin fraction with ellagitannin content of 80%. This may be caused by activation of the efflux mechanism. Also, ketoprofen permeability was clearly decreased by the ellagitannin fraction in a dose-dependent manner, hence it could stay longer in the intestine causing some of the adverse effects reported (Abebe 2002). On the contrary, the anthocyanin fraction increased the permeability of ketoprofen and verapamil in a dose-dependent manner but the effect was not so clear as with the ellagitannin fraction. High TEER values after the experiments indicate

Table 2 Anti-mutagenicity activity of raspberry samples as R-values showing the amount of colonies formed in comparison with HBSS (B) or S9 control

Samples	TA98 + 2N + B	TA98 + 2A + S9	TA100 + Na + B	TA100 + 2A + S9
Acetone extract	2.5	4.9	3.7	6.5
Ellagitannins	2.2	4.9	3.4	6.4
Anthocyanins	2.2	5.0	3.3	6.3
Control	5.6	5.2	3.8	4.8

Salmonella typhimurium strains TA98 and TA100 without metabolic activation (B), with metabolic activation (S9), 2-nitrofluorone (2N), 2-aminoanthracene (2A), HBBS buffer (B), sodium azide (Na).

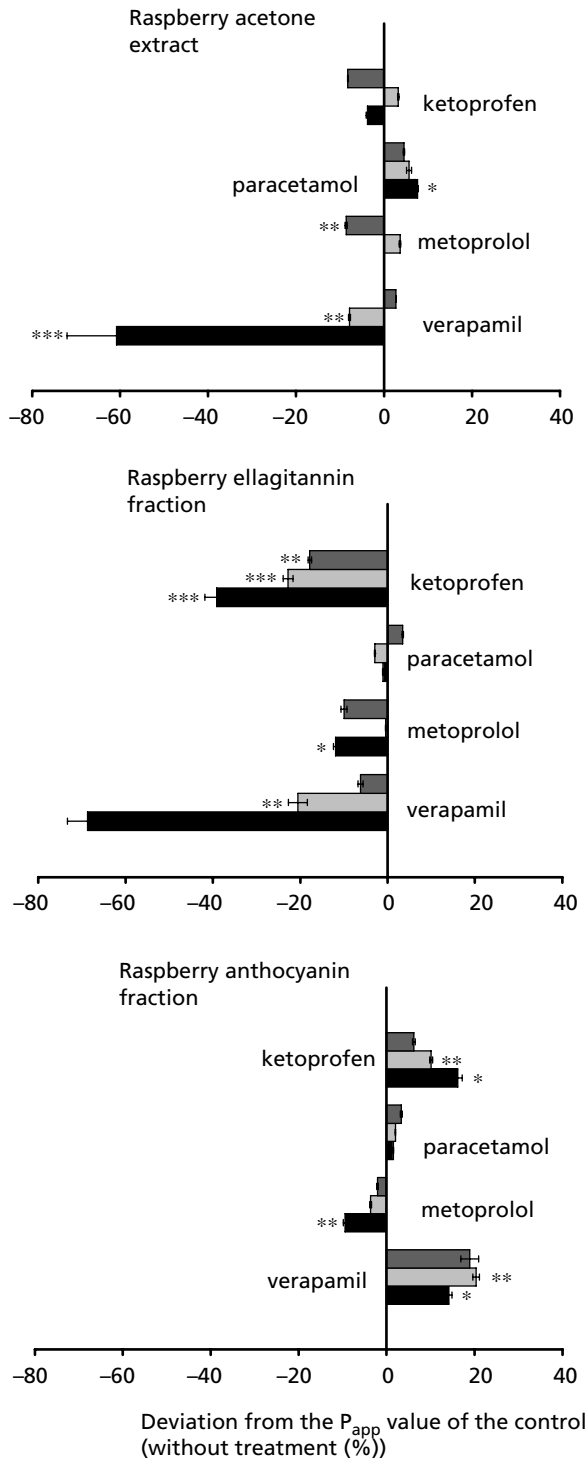

Figure 1 Changes in permeabilities of ketoprofen, paracetamol, metoprolol and verapamil in the presence of various concentrations of raspberry acetone extract, ellagitannin fraction and anthocyanin fraction (1.0; 0.1; 0.01 mg mL^{-1}) across Caco-2 cell monolayers. Values are mean percentage differences ± s.d., n = 3, from the permeabilities of the compounds across Caco-2 cell monolayers in the absence of the extracts (control values). Black bars represent the strongest, light grey bars the medium and dark grey bars the mildest concentration of the studied extracts. $*P < 0.1$, $**P < 0.05$, $***P < 0.01$ vs control.

that the paracellular spaces were not affected. Permeability of paracetamol was not affected by any of these compounds.

Interestingly, the drugs showed similar permeability profiles when co-administered with the raspberry extract as with the ellagitannin fraction, whereas the anthocyanin fraction caused a different permeability behaviour for the studied drugs. This clearly shows that when components are used in more concentrated form, adverse effects may occur even though milder mixture of the same components lack effects. In some cases effects might disappear. For example, a complex mixture of substances may possess activity even though the single compounds lack efficacy. This is why different products have to be examined case by case and no generalization should be made. In our earlier study (Laitinen et al 2004) both the glycosidic and aglyconic raspberry extracts exhibited only minor effects. Effects could only be combined with changes in pH levels in samples where the pH was not adjusted. For many plant products, with potential for drug product or functional food development, the polyphenol composition is much less known than in this study. Also numerous factors other than variety may affect the polyphenol content of plants; these factors include ripeness at the time of harvest, environmental factors, processing and storage. The difference in the used varieties, as well as the different preparation of the raspberry extracts, explains the differences in the behaviour in this study and our earlier study, further indicating the importance of chemically specified samples.

Conclusions

Diets or supplements containing phytochemicals and other bioactives will certainly help in preventing many chronic diseases, but there are still many important scientific questions that need to be answered. Studies such as standardization of methods for quantification, evaluation of toxic and mutagenic effects in addition to found bioactivity, bioavailability and interactions of food components with drugs need to be conducted.

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