Eur J Nutr 38:227–234 (1999)

ORIGINAL CONTRIBUTION

B.L. Pool-Zobel
A. Bub
N. Schröder
G. Rechkemmer

Anthocyanins are potent antioxidants in model systems but do not reduce endogenous oxidative DNA damage in human colon cells

Received: 28 July 1999
Accepted: 8 October 1999

Prof. Dr. B.L. Pool-Zobel (✉)
Friedrich-Schiller-University of Jena
Department of Nutritional Toxicology
Institute for Nutrition
Dornburger Str. 25
D-07743 Jena
Germany
e-mail: b8pobe@uni-jena.de

A. Bub · G. Rechkemmer
Institute of Nutritional Physiology
Federal Research Centre for Nutrition
Haid-und-Neu Str. 9
D-76131 Karlsruhe
Germany

N. Schröder
Dr. Rainer Wild Stiftung
In der Aue 4
D-69118 Heidelberg-Schlierbach
Germany

Summary Anthocyanins are common colored plant flavonoids, occurring as glycosides of the respective anthocyanidin-chromophores. Like other flavonoids, anthocyanidins are also expected to have antioxidative and antimutagenic properties *in vivo*, although only few data are available. To gain more knowledge on possible protective mechanisms in mammalian cells, we have compared their extracellular and intracellular antioxidative potential *in vitro* and in human colon tumor cells. We used *Aronia melanocarpa* Elliot anthocyanin (AA) concentrates, fractions thereof, concentrates from Elderberry, Macqui, and Tintorera fruits, as well as pure compounds. *In vitro*, antioxidative properties of the samples were studied with the ferric reducing ability assay (FRA assay). As a measure of intracellular oxidative/antioxidative effects, H_2O_2-induced strand breaks as well as oxidized DNA bases were determined in human tumor HT29 clone 19A cells using a microgelelectrophoresis assay (comet test). Major results were that isolated compounds (aglycons and glycosides) and complex plant samples are powerful antioxidants *in vitro*. In fact their activities by far exceeded those of Trolox and vitamin C in the FRA assay. Also, H_2O_2-induced DNA strand breaks were reduced in cells treated with the complex plant extracts. In contrast, endogenous generation of oxidized DNA bases was not prevented. In summary, the intracellular steady state of oxidized DNA bases is not altered by anthocyanins or anthocyanidins. This finding raises questions with respect to the cancer preventive potential of anthocyanidins within specific tissues, such as the colon. Extracellularly, however, the compounds are potent antioxidants. This points to their potential for providing systemic protection *in vivo*, e.g., by scavenging oxidants in the blood stream and in the colon. Notably, both aglycons and glycosides have equally strong antioxidant activity.

Key words Anthocyanidins – anthocyanins – comet assay – antioxidative potential – oxidized DNA-bases

Introduction

Flavonoids are implicated in preventing diseases related to oxidative stress, such as coronary heart afflictions and cancer (1, 2). The glycoside anthocyanins and their aglycon anthocyanidins – blue and red flavonoids found in many berries, dark grapes, cabbage, and other red colored plant foods – may contribute to cancer preventive potentials of fruits and vegetables via antioxidant mechanisms, although the epidemiological evidence is insufficient (3, 4). Some anthocyanidins and other flavonoids of grapes are antioxidative *in vitro* by scavenging reactive oxygen species (5, 6), by being involved in metal chelation (7)

and by inhibiting lipoprotein oxidation (8–10). Thus, systemic antioxidative effects by circulating anthocyanidins in body fluids are expected to reduce the body's load of oxidants and ultimately the risk for developing the corresponding diseases. For cancer development, however, it is not resolved to what extent antioxidative protection exerted by anthocyanins/anthocyanidins is actually present in the target cell of tumorigenesis. Protection during tumor initiation and progression should be enhanced by intracellular inhibition of oxidative processes leading to DNA damage. Only a few reports are available for the activities of anthocyanidins within isolated cells. One study by Duthie et al. shows that the flavonoids quercetin and myrecetin induce DNA damage and inhibit proliferation in various human cell types at concentrations above 100 µM (11). Virtually no information is available on the bioactivity of the corresponding anthocyanins, which are the forms present in the plant foods and represent the majority of compounds which are actually ingested.

Oxidized bases are a result of the oxidative stress prevailing within cells and are very important types of early genetic damage, which may be involved during carcinogenesis (12). Both processes of initiation and progression are expected to be enhanced as a result of oxidative DNA damage (13, 14). Recently, a new method was developed with which it is possible to determine oxidized DNA bases within single cells (15, 16). Using this technique (the comet assay, or single cell microgelelectrophoresis assay), we observed a reduction of oxidized DNA bases of approximately 50% in human lymphocytes subsequent to antioxidative vegetable consumption during a human dietary intervention study (percent intensity of fluorescence in comet tail [%TI] was 21±4 and 9±1 before and after intervention, respectively; means ± SEM, n=21) (17, 18). Recently we have also been able to determine the variable levels of oxidized bases in human colon cells (19, 20). Moreover, preliminary investigations with primary human colon cells isolated from biopsies have shown that preincubation with a concentrate of *Aronia melanocarpa* ELLIOT (black chokeberry) containing anthocyanidins and anthocyanins was able to reduce the H_2O_2-induced levels of DNA damage by 50% (21).

In order to determine intracellular antioxidative capacity of these colored flavonoids, we investigated anthocyanin-enriched concentrates of *Aronia melanocarpa*, subfractions thereof, and the major ingredients cyanidin, idaein, and cyanin. Similarly, concentrates of elderberry, macqui, and tintorera grape were investigated. Specifically, human colon tumor cells HT29 clone 19A were incubated with the samples and the formation of these oxidized bases was determined. These intracellular effects are compared with the basic *in vitro* antioxidant properties (in the absence of cells) determined with the ferric reducing ability assay (FRA assay) (22).

Material and methods

Preparation of anthocyanin concentrates

Fruit juices containing anthocyanins and anthocyanidins were prepared from fresh berries of aronia (*Aronia melanocarpa*), elderberry (*Sambucus nigra),* macqui *(Aristotelia chilensis*), and the tintorera grape by common methods of fruit juice preparation. They were concentrated *in vacuo* and stored until further processing at -70 °C. For anthocyanin enrichment, these fruit concentrates were diluted in demineralized water to 15° Brix. 100 g of the juices were loaded onto a chromatography column (diameter 5 cm/ length 20 cm, filled with 170 g RPMI18 material). The column was rinsed with 0.5 l water. The adsorbed materials were released by a mixture of ethanol/water (1:1, v/v). The pH of the eluent was adjusted to two with hydrochloric acid. All solutions were concentrated *in vacuo* at 60 °C to yield the final anthocyanin concentrates (Table 1) which were further stored at -70 °C under nitrogen atmosphere until use. The degree of concentration was twofold, e.g., 2 kg *Aronia melanocarpa* fruit containing 16 g anthocyanins/anthocyanidins were concentrated to yield a volume of 1000 ml anthocyanin concentrate. The samples were analyzed by the FRA assay and the comet assay.

Preparations of purified fractions from *Aronia melanocarpa* concentrates

The anthocyanin concentrate from 2 kg *Aronia melanocarpa fruit* was further fractionated by preparative column chromatography. Analysis of the complex factions revealed peaks which co-chromatographed with authentic

Table 1 Anthocyanin concentrations in enriched samples from different fruits

Sample	BRIX	pH value	absorbance at pH3 (diluted 1:1000)	anthocyanin concentration mg/kg	Amount (µl) added to 1ml*
Aronia	16.7	0.89	0.453	16016	1.56
Macqui	24.4	0.86	0.617	30090	0.83
Elderberry	19.6	3.12	0.526	17745	1.41
Grape	14.3	1.83	0.313	14973	1.67

* This amount gives 25 µg anthocyanins/anthocyanidins in 1 ml

samples (23). Freeze drying of the aqueous and alcoholic fractions resulted in a yield of 1.7 g of fraction 1 (containing mainly phenolic compounds; λ max. 320 nm), 1.1 g of fraction 2 (containing mainly anthocyanins/anthocyanidins), and 0.1 g of fraction 3 (containing heterogeneous other compounds with longer retention times). The relative yields were 59%, 38%, and 3% for fractions 1, 2, and 3, respectively. Fraction 2 consisted of both cyanidin-anthocyanins as well as the aglycon cyanidin (Table 2). Fractions 1 and 3 were basically anthocyanidin- and anthocyanin-free.

Table 2 Anthocyanin and cyanidin content in fraction 2 isolated from the anthocyanin concentrate of *Aronia melanocarpa*

Compound	*Content mg/g*
Cyanidin -3-galactosid	166
Cyanidin-3-glucoside	6
Cyanidin-3-arabinoside	39
Cyanidin-3-xyloside	4
Cyanidin	89

Dosing of the complex samples for the *in vitro* assays

The doses of the anthocyanidin concentrates were chosen to yield multiples of 25 µg anthocyanins/anthocyanidins in 1 ml. The amounts in the concentrates were estimated on the basis of absorbance at pH 3. The calculated amount of concentrate added to 1 ml to give 25 µg/ml is shown in the final column of Table 1. Thus portions of, e.g., 1.56–7.8 µl of the Aronia concentrate are equivalent to 25–125 µg anthocyanins in 1 ml incubation mixture for both the FRA and comet assays. On the basis of an average molecular weight of 250, this is very roughly equivalent to 100–500 µM.

The doses of the isolated fractions were chosen on a different basis. In the FRA assay they were investigated in portions of 10 to 100 µl/ml. For the assays with colon cells the amounts added to 1 ml cell suspension were based on the relative yield of 58:38:3 for fractions 1, 2, and 3, respectively. Thus, to test 25 µg anthocyanin/anthocyanidin equivalents, fraction 2 (which consists basically of 100% anthocyanidins and cyanidin, Table 2) was added in amounts of 25 µl/ml. Correspondingly 38.63 µg/ml of fraction 1 and 2 µg/ml of fraction 3 were added to yield 1 ml final suspension culture. The relation of 38.63:25:2 µl is equivalent to the relative yields of 58:38:3 % (see above).

Antioxidant activity assay

To measure antioxidant activity, the ferric reducing ability (FRA) assay was used as described by Benzie and Strain with minor modifications (22). In brief, FRA reagent was prepared as required by mixing 25 ml acetate buffer (300 mM), 2.5 ml 2,4,6-tripyridyl-s-triacine (TPTZ, 10 mM, Fluka Chemicals, Switzerland), and 2.5 ml $FeCl_3$.$6H_2O$ (20 mM Merck, Darmstadt, Germany). Aqueous solutions of $FeCl_3$ concentrations between 100–1000 µM were used for calibration. In a 96 well microtiter plate, unknown samples (diluted with NaCl to yield 10 µl portions) were added to 30 µl H_2O and the reaction was started by further addition of 300 µl prewarmed (30 °C) FRA reagent. The reaction mixture was incubated for 8 min at 30 °C and absorbance at 585 nm was determined in a microtiter plate reader (MWG Biotech, Ebersberg, Germany). H_2O instead of sample served as reagent blank. Each assay was reproduced independently at least 3 times. Intra- and inter-assay variance were less than 5%.

Treatment of cells with chemicals

Compounds and complex samples were diluted with or dissolved in 10% DMSO in 0.9% NaCl. The solutions were added in amounts of 10 µl per ml cell suspension containing 2 x 10^6 HT29 clone 19A cells (24, 25). The suspensions were incubated for 15 minutes in a shaking water bath at 37 °C or statically on ice at 4 °C, and then centrifuged. This treatment period had previously been shown to be effective in reducing intracellular levels of oxidized bases in primary human colon cells (21). The pellet was taken up in agarose, distributed onto slides and then processed according to the protocol for the microgelelectrophoresis as described below.

H_2O_2 was dissolved in 0.9% NaCl. The treatment with H_2O_2 was performed by incubating the slides covered with cells embedded in agarose with 50 µl of solutions containing different concentrations of H_2O_2 (12.5 µM–100 µM) for 5 minutes on an ice cold rack, to avoid repair of the rapidly induced oxidative DNA damage (26). The cells were then placed in a lysis bath and all other steps proceeded as described below.

Determination of genetic damage

Ten microliter of the cell suspensions (containing 2x10^5 HT29 cells or 3–4 x 10^5 primary human colon cells) was mixed with 75 µl 0.7% low melting point agarose and distributed onto microscope slides coated with 0.5% normal melting agarose. After solidification of the agarose, slides were covered with another 75 µl 0.7% low melting point agarose and then immersed in a lysis solution (100 mM Na_2EDTA, 1% Triton X 100, 2.5 M NaCl; 1% N-lauroyl sarcosin sodium salt, 10% DMSO, 10 mM Tris pH 10) for at least 60 minutes. Slides being processed for oxidized bases were washed and incubated with endonuclease III or formamidopyrimidine glycosylase in buffer sealed with a cover slip for 45 minutes at 37 °C. All slides were placed in an electrophoresis chamber containing alkaline solution (1 mM Na_2EDTA, 300 mM NaOH, pH 13) for

DNA unwinding. After 20 minutes the current was switched on and electrophoresis was carried out at 25 V, 300 mA for 20 minutes. The slides were removed from the alkali and washed 3 times 5 minutes with neutralization buffer (0.4 M TRIS, pH 7.5). Slides were stained with ethidium bromide (20 µg/ml; 100 µl/slide). All steps beginning with the isolated cells were conducted under red light. For each data point and experiment two gels were processed with endonuclease III (kind gift of A. Collins, Aberdeen U. K.), two with formamidopyrimidine glycosylase (kind gift of B. Epe, Mainz, Germany), and two slides without enzymes for determination of oxidized pyrimidine bases, oxidized purine bases, and DNA strand breaks, respectively. The experiments were independently reproduced at least 6 times.

Evaluation of the microgelelectrophoresis assay

Microscopical evaluation of the images was quantified using the image analysis system of Perceptive Instruments (Halstead, UK). Fifty images were evaluated per slide and the percentage of fluorescence in the tail (TI, "tail intensity") was scored. The "tail intensity yield" (values of slides treated with endonuclease III minus values from corresponding slides without the enzyme) is a reflection of the levels of oxidative DNA damage.

Statistical analysis

Prism software (Graph Pad) was used for establishing two sided significance levels. As is specified in the figures, based on the means of triplicate parallel determinations, paired and unpaired *t-tests* were calculated as appropriate.

Miscellaneous other chemicals and reagents

Hydrogen peroxide (Cas No. 7722-84-1) was obtained as a 30% aqueous solution from Merck, Darmstadt, Germany. Protease and collagenase were from Amresco, Solon, Ohio, U.S.A., and Boehringer, Mannheim, Germany, respectively. Dulbecco's modified minimal essential medium (DMEM) was from Gibco BRL, Eggenstein, Germany. All other chemicals were of analytical grade or complied with the standards needed for tissue culture experiments. Cyanidin chloride, cyanin chloride (cyanidin chloride-3,5-diglucoside), and idaein chloride (cyanidin chloride-3-galactoside) were commercially available from Carl Roth GmbH & Co. KG, Karlsruhe, Germany.

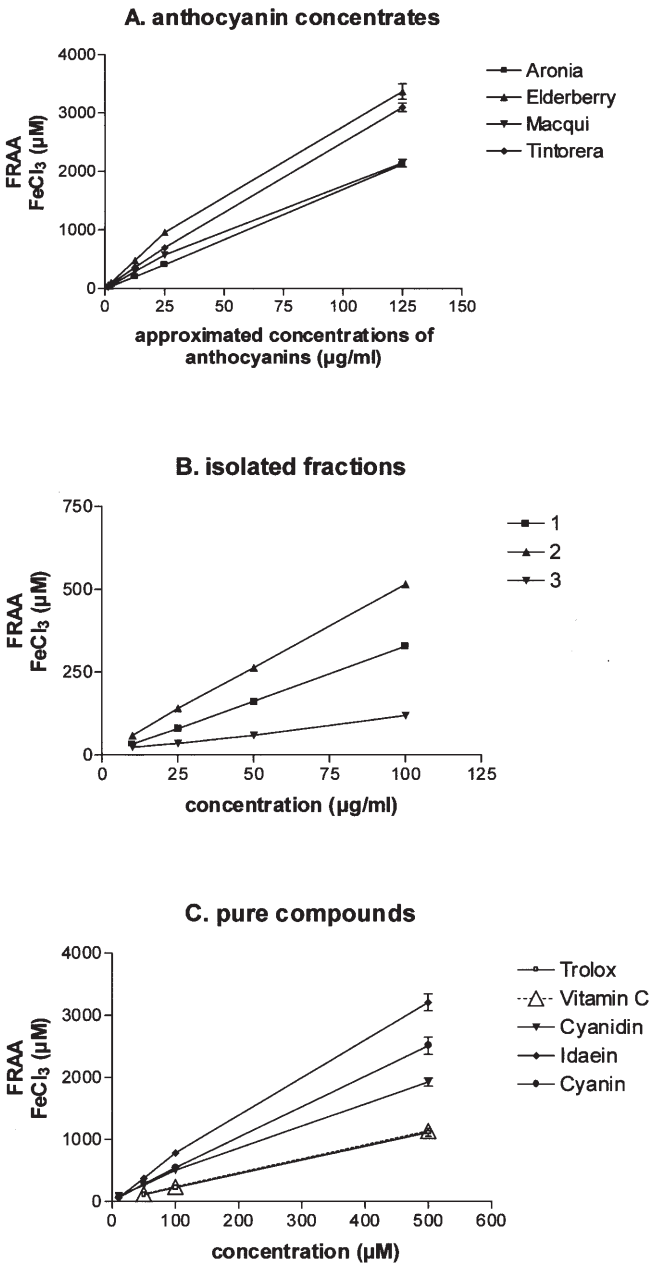

Fig. 1 Activities of anthocyanin concentrates (A), isolated fractions from the anthocyanin concentrates (B), and pure compounds (C) in the FRA assay (FRAA). Shown are means ± SD of 3 individual determinations (A, C), with the exception of (B) for which only one determination was possible due to limited supply of samples.

Results

Aronia melanocarpa anthocyanidin concentrates and isolated fractions were potently active as *in vitro* antioxidants in the FRA assay (Fig. 1A–B). Also, the pure compounds were active as well (Fig. 1C). In fact for 100 µM of the pure compounds the antioxidant activity was 2- to 5-fold higher than equimolar concentrations of ascorbic acid or the water soluble vitamin E analogue Trolox which were both used as reference compounds (Fig. 1C). The antioxidant activity of the aglycon cyanidin was less than that of the glycosides, cyanin, and idaein (Fig. 1C).

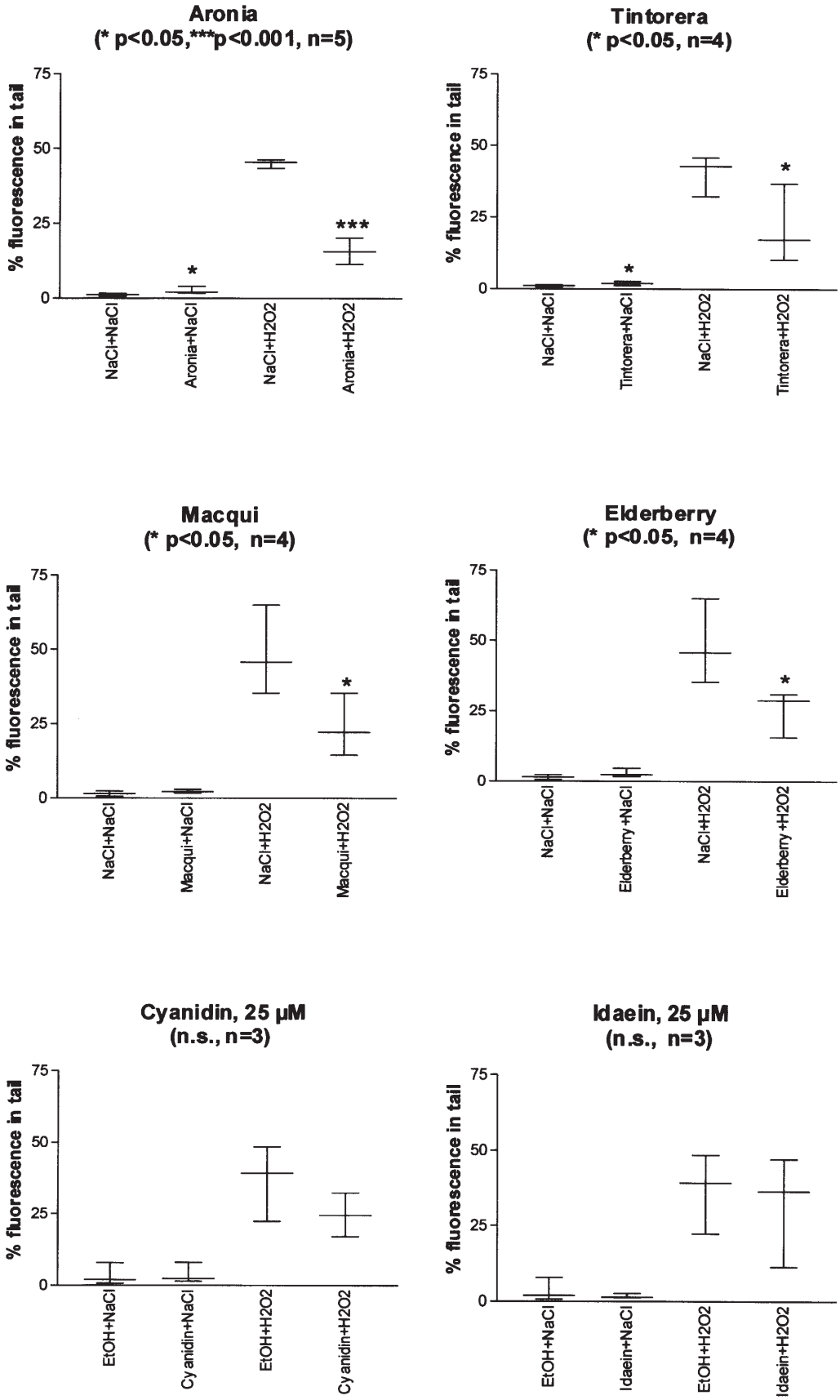

Fig. 2 Box and whisker plots on the DNA damaging activity of H_2O_2 (4C 5' treatment on slide) in control and anthocyanin/anthocyanidin-treated cells (37 °C, 15' in tube). Significant differences between and anthocyanins/anthocyanidin-treated samples were determined with a two-sided unpaired t-test.

Among the anthocyanin concentrates elderberry exhibited the strongest antioxidant activity followed next by tintorea and subsequently by aronia and macqui (Fig. 1A).

Purification and fractionation of *Aronia melanocarpa* concentrates revealed 3 fractions which were also tested for their antioxidant potential (Fig. 1B). Fraction 2, containing anthocyanins and cyanidin, was most effective.

Antioxidant-like effects on human cells were also observed for the four anthocyanin concentrates, in that they were able to prevent H_2O_2 induced DNA damage in HT29 clone 19A colon cells. No significant effects were observed with the isolated, pure compounds, although there was a trend for an effect with the aglycon cyanidin (Fig. 2). Also, Fig. 2 shows that under these experimental conditions a slight genotoxic activity is observed for the aronia and tintorera samples, which, however, was not reproducible in another independent set of experiments (Fig. 3). Cyanidin and idaein are only marginally genotoxic at 100 µM (Fig. 3).

Using these dilutions with low genotoxic activity as maximal concentrations, the complex mixtures and compounds were subsequently investigated for their ability to reduce endogenous oxidative stress. This was achieved by incubating the cells with the test substances and then determining endogenously formed oxidized DNA bases using repair specific enzymes. Figure 4 shows the results for the prevention of oxidation of purine bases. The anthocyanin concentrates do not have a potential to protect

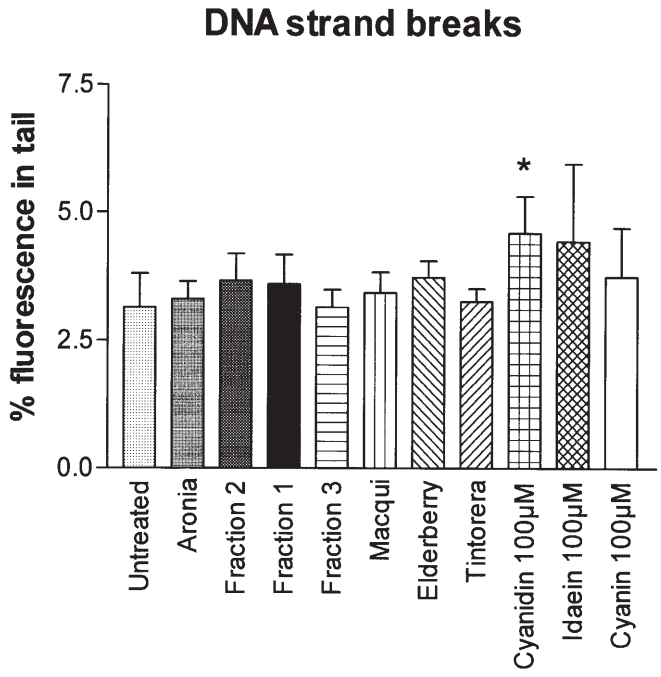

Fig. 3 Induction of DNA strand breaks by anthocyanins and anthocyanidins in HT29 clone 19A cells (*p < 0.05, two tailed paired *t*-test; n=7).

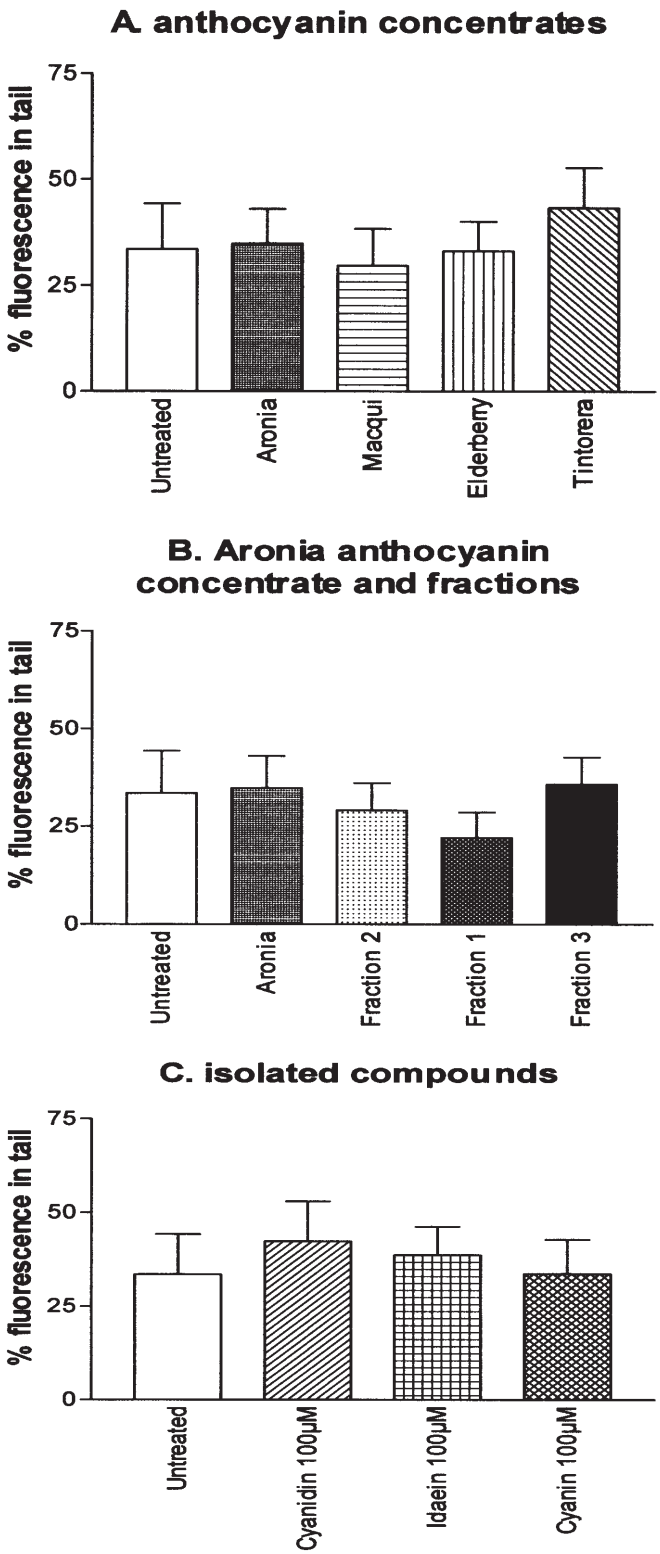

Fig. 4 Modulation of steady-state levels of oxidized purine bases in HT29 clone 19A cells following treatment with anthocyanin/anthocyanidin concentrates of different fruits (A), the anthocyanidin concentrate of aronia and isolated fractions thereof (B), and with individual anthocyanins and cyanidin (C; 100 µM); (n.s., two tailed unpaired *t*-test; n=6).

the cells against the development of oxidized purine bases. The same is true for the anthocyanidin containing fraction 2 of the aronia concentrate (Fig. 4B) and for the major anthocyanins and anthocyanidins present in fraction 2 (Fig. 4C). All tested samples were also ineffective at modulating oxidized pyrimidine bases, with the exception of the elderberry anthocyanidin-concentrate which had a marginal effect (results not shown). Idaein and cyanidin were also investigated at lower concentrations (25 and 50 µM; results not shown), but had no effects on the steady-state level of oxidized DNA bases (results not shown).

Discussion

Flavonoids in particular, and at times also anthocyanins and anthocyanidins, have been a focus for discussions pertaining to beneficial properties of red wine, and other non-alcoholic beverages and food (8, 27). On account of their antioxidative potentials, they have been implicated in reducing the risk for coronary heart disease, cancer, and macula degeneration (28–30). Our studies indeed point to a particular efficacy of these compounds to act as antioxidants in body fluids and are in line with *in vivo* observations of human studies (31). The results of the FRA assay clearly indicate a high potential of plant extracts enriched with these particular compounds to act as antioxidants. The mechanisms of antioxidative effects are, however, not clear. In this FRA test, the antioxidative capacity may be a reflection of the compounds', potential to chelate $FeCl_3$, as well as to scavenge free radicals. Interestingly, whatever the mechanisms, the activities are not only due to the aglycon (cyanidin) but more so to the corresponding glycosides, cyanin, and idaein. These are also expected to be the major compounds of the potent antioxidative-plant extracts, concentrates, and fractions. Glycosides are expected to be decomposed by ß-glycosidase activity in the gut flora. Thus, a resorption of the intact glycosides before reaching the intestines may lead to a better plasma antioxidant capacity (32). This is potentially the mechanism of importance for counteracting adverse accumulation of endothelial depositions (27). In this context, important are recent studies which show that glycosides of quercetin, consumed with an onion meal, are indeed well absorbed and present in the blood stream (33). Therefore, not only aglycons but also glycosides are of high physiological relevance.

Another mode of protecting cells may be the extracellular reduction of exposure to oxidative and carcinogenic factors. Thus the finding that the crude plant concentrates protect colon cells against H_2O_2-damage is of importance in this respect. This, observation has also been made using primary human cells, which are even more relevant surrogates of colon tissue than the cultured HT29 clone 19A cell line (21). These antioxidative effects may be due to the anthocyanidins, since cyanidin did show a trend of protection against H_2O_2 induced damage (Fig. 2). However, since idaein was ineffective, the protection by the concentrates may very well be due to other antioxidative compounds in the complex samples. In any case, the mechanism is unspecific and therefore will probably be of importance *in vivo* for virtually any type of tissue. In contrast, the group of test samples did not show cell-specific modes of protection, since they did not affect steady-state levels of oxidized DNA bases in cultivated colon cells. With few exceptions neither oxidized purine nor pyrimidine bases were reduced in the cells treated with the test samples.

In conclusion, intracellular oxidative stress is only weakly affected by the natural plant ingredients anthocyanins/anthocyanidins. However, these compounds are potent extracellular antioxidants and may also protect cells against external oxidative species. An important finding of this study is that the glycosides are at least as or even more effective than the aglycons in the FRA assay.

Acknowledgments We gratefully acknowledge Mr. M. Knoll, Ms. R. Lambertz, Ms. B. Mathony-Holschuh, and Ms. C. Stoltz for excellent technical assistance. We are very much obliged to the "Dr. Rainer-Wild-Stiftung" for financing this research, to the "Wild Werke" Heidelberg for the cooperative input in providing the anthocyanidin concentrates, and fractions, as well as for characterizing the ingredients, to Dr. A. Collins, Aberdeen, UK for the kind gift of endonuclease III, and to Prof. Dr. B. Epe, Mainz, for formamidopyrimidine glycosylase.

Reference List

1. Hertog MGL, Feskens EJM, Hollman PCH, Katan MB (1993) Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen Elderly Study. The Lancet 342:1007–1011
2. Stoner GD, Mukhtar H (1995) Polyphenols as cancer chemopreventive agents. Journal of Cellular Biochemistry, Supplement 22:169–180
3. World Cancer Research Fund and American Institute for Cancer Research (1997) Food, Nutrition and the Prevention of Cancer: a Global Perspective, American Institute for Cancer Research, Washington DC
4. Goldbohm RA, Hertog MGL, Brants HAM, van Poppel G, van den Brandt PA (1998) Intake of flavonoids and cancer risk: a prospective cohort study. InCOST 916 Bioactive Plant Cell Wall Components in Nutriution and Health: Polyphenols in Food (Ed. EU 181689), pp 166
5. Yamasaki H, Uefuji H, Sakihama Y (1996) Bleaching of the red anthocyanin induced by superoxide radical. Archives Biochemistry Biophysics 332: 183–186
6. Tsuda T, Ohshima K, Kawakishi S, Osawa T (1996) Oxidation products of cyanidin 3-O-ß-glucoside with a free radical initiator. Lipids 31:1259–1263
7. Kühnau J (1976) The flavonoids. A class of semi-essential food components: their role in human nutrition.

World Review on Nutrition and Diet 24:117–191
8. Ghiselli A, Nardini M, Baldi A, Scaccini C (1998) Antioxidant activity of different phenolic fractions separated from an Italian red wine. J Agric Food Chem 46:361–367
9. Tsuda T, Shiga K, Ohshima K, Kawakishi K, Osawa T (1996) Inhibition of lipid peroxidation and the active oxygen radical scavenging effect of anthocyanin pigments isolated from *Phaseolus vulgaris* L. Biochem Pharmacol 52:1033–1039
10. Kerry NL, Abbey M (1997) Red wine and fractionated phenolic compounds prepared from red wine inhibit low density lipoprotein oxidation in vitro. Atherosclerosis 135:93–102
11. Duthie SJ, Johnson W, Dobson VL (1997) The effect of dietary flavonoids on DNA damage (strand breaks and oxidised pyrimidines) and growth in human cells. Mutat Res 390:141–151
12. Ames BN, Shigenaga MK, Hagen TM (1993) Oxidants, antioxidants, and the degenerative diseases of aging. Proc Natl Acad Sci USA 90:7915–7922
13. Cerutti PA (1985) Prooxidant states and tumor promotion. Science 227: 375–381
14. Dreher D, Junod AF (1996) Role of oxygen free radicals in cancer development. European Journal of Cancer 32A:30–38
15. Collins AR, Duthie SJ, Dobson VL (1995) Direct enzymic detection of endogenous oxidative base damage in human lymphocyted DNA. Carcinogenesis 14:1733–1735
16. Collins AR, Dusinská M, Gedik CM, Stetina R (1996) Oxidative damage to DNA: Do we have a reliable biomarker? Environ Health Perspect 104:465–469
17. Pool-Zobel BL, Bub A, Müller H, Wollowski I, Rechkemmer G (1997) Consumption of vegetables reduces genetic damage in humans: first results of an intervention trial with carotenoid-rich foods. Carcinogenesis 18:1847–1850
18. Pool-Zobel BL, Bub A, Liegibel UM, Treptow-van Lishaut S, Rechkemmer G (1998) Mechanisms by which vegetable consumption reduces genetic damage in humans. Canc Epid Biom Prev 7:891–899
19. Pool-Zobel BL, Bub A, Rechkemmer G (1997) Application of the COMET assay to study oxidative DNA damage in human cells. In: Antioxidant Methodology: In Vivo and In Vitro Concepts. Anonymous AOCS Press, Champaign, pp 39–52
20. Pool-Zobel BL, Abrahamse SL, Collins AR, Kark W, Gugler R, Oberreuther D, Siegel EG, Treptow-van Lishaut S, Rechkemmer G (1999) *Analysis of DNA strand breaks, oxidized bases and glutathione S-transferase P1 in human colon cells*. Canc Epid Biom Prev 8: pp 609–614
21. Rechkemmer G, Pool-Zobel BL (1998) Antigenotoxic and physiological properties of anthocyanins/anthocyanidins in intestinal epithelial cells. In: COST 916, Bioactive plant Cell Wall Components in Nutrition and Health: Polyphenols in Food (EUR 18169), pp 131–136
22. Benzie IFF, Strain JJ (1996) The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Analyt Biochem 239: 70–76
23. Weinges K, Schick H, Schilling G, Irngartinger H, Oeser T (1997) Composition of an anthocyan concentrate from *Aronia melanocarpa* Elliot - X-ray analysis of tetraacetyl parasorboside. European Journal of Organic Chemistry 1998:189–192
24. Augeron C, Laboisse CL (1984) Emergence of permanently differentiated cell clones in a human colonic cancer cell line in culture after treatment with sodium butyrate. Cancer Res 44: 3961–3969
25. Rousset M (1986) The human colon carcinoma cell lines HT-29 and Caco-2: two in vitro models for the study of intestinal differentiation. Biochimie 68:1035–1040
26. Collins AR, Ai-guo M, Duthie SJ (1995) The kinetics of repair of oxidative DNA damage (strand breaks and oxidized pyrimidines) in human cells. Mutation Research 336:69–77
27. Frankel EN, Kanner J, German JB, Parks E, Kinsella JE (1993) Inhibition of oxidation of human low-density lipoprotein by phenolic substances in red wine. The Lancet 341:454–457
28. Hoey J, Montvernay C, Lambert R (1981) Wine and tobacco: risk factors for gastic cancer in France. American Journal of Epidemiology 113:668–674
29. Gilman MW, Cupples LA, Gagnon D, Posner BM, Ellison RC, Castelli WP, Wolf PA (1995) Protective effect of fruits and vegetables on development of stroke in men. J Am Med Assoc 273:1113–1117
30. Renaud S, DeLorgeril M (1992) Wine, alcohol, platelets, and the French paradox for coronary heart disease. The Lancet 339:1523–1526
31. Whitehead TP, Robinson D, Allaway S, Syms J, Hale A (1995) Effect of red wine ingestion on the antioxidant capacity of serum. Clinical Chemistry 41:32–35
32. Hollman PCH, van Trijp JMP, Mengelers MJB, deVries JHM, Katan MB (1997) Bioavailability of the dietary antioxidant flavonol quercetin in man. Cancer Lett 114:139–140
33. Aziz AA, Edwards CA, Lean MEJ, Crozier A (1998) Absorption and excretion of conjugated flavonols, including quercetin-4'-O-ß-glucoside and isohamnetin-4'-O-ß-glucoside by human volunteers after the consumption of onions. Free Rad Res 29:257–269