Antioxidant capacity of human plasma and serum as affected by a single dose of a beverage rich in antioxidants – use of three different assay systems

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Abstract
A single dose of polyphenols comprised in a beverage based on fruit juices did not significantly increase the content of total phenolics neither in plasma nor in serum. How-ever, the ingestion of 500 mL beverage significantly enhanced the antioxidant capacity in plasma (FRAP) as well as in serum (FRAP, TRAP), reaching the peak after different times (30-60 min). In addition, the photochemiluminescence test system, being a very sensitive assay in recent investigations, did not show any alteration in antioxidant capacity neither in plasma nor in serum. Thus, the kind of physiological liquid a well as the kind of test system seem to be factors affecting the analysis of antioxidant capacity within human intervention studies. The use of at least two assays is strongly recommended.

Key words: Antioxidant capacity, human plasma, human serum, total phenolics, FRAP assay, TRAP assay.

Introduction
Several epidemiological studies suggest the importance of a high consumption of secondary plant products in reducing the incidence of degenerative diseases like cancer and arteriosclerosis1. These substances, widely distributed in fruits and vegetables, possess a high anti-oxidative potential. A large number of analytical methods have been used to assess the protective effect in human plasma after ingestion of e.g. red wine or tea2-5. Critical com-parisons of 3-6 in vitro methods were done by analyzing reference substances or plant products. Only scarce results exist for comparison of different test systems analyzing physiological fluids for their antioxidant potential. Cao et al. analyzed human serum and urine by using three methods (Trolox Equivalent Antioxidant Capacity (TEAC), Ferric Reducing Ability of Plasma (FRAP), Oxygen-Radical Absorbing Capacity (ORAC)). The same three methods were used in another trial for analysis of human serum and plasma for their antioxidant capacity. The aim of this study was to evaluate the effect that a single dose intervention with a beverage rich in polyphenols has on the antioxidant capacity, determined in human plasma and serum by using three different methods. In addition, the content of total phenols as parameter summarizing the ingested poly-phenols was to be determined. The content of uric acid, the most important physiological antioxidant in blood, had to be analyzed for control purposes.

Materials and Methods

Participants and study protocol: Five healthy male volunteers took part in this pilot study, all non-smokers and not taking any medication or vitamin supplements. Their main characteristics are shown in Table 1. All participants gave their informed written consent. The study protocol was approved by the Local Ethical Committee. All persons ingested 500 mL of a beverage rich in antioxidants together with a standardized breakfast (2 wheat rolls, 20 g butter, 50 g jam). The beverage was based on apple juice, grape juice, lemon juice, elderberry juice, and black currant juice, with 30% juice content in total. Further ingredients were extracts of green tea (2.28 g/L), sage (0.57 g/L), rosemary (0.38 g/L), and hawthorn leaves and blossoms (0.05 g/L) (total amount of extract 3.3 g/L; total phenolics content (gallic acid equivalents, GAE, of extracts 878 mg/L, GAE of test beverage 1283 mg/L). The beverage was produced with the intention to combine a variety of antioxidative plant polyphenols from different fruits and herbs in order to produce a food with high antioxidant activity. Blood samples were drawn from the study particpants in serum tubes without anticoagulant and EDTA tubes prior to ingestion of the beverage and 30, 60, 90 and 120 min after ingestion.

Analytical methods including sample preparation: Immediately after withdrawing, the plasma was separated by centrifugation (1000 g, 20°C). Prior to serum separation by centrifugation (1800 g, 20°C), blood was allowed to clot at room temperature for 30 min. Plasma and serum samples were stored at -80°C until analysis. Uric acid was determined spectrophotometrically (540 nm) by using a commercial kit (Merck, Darmstadt, Germany). Total phenolics were analyzed spectrophotometrically (750 nm) by using a modified Folin-Ciocalteu method. Sample preparation included hydrolysis of conjugated polyphenols by using hydrochloric acid, saponification with methanolic sodium hydroxide solution, precipitation of proteins with meta phosphoric acid, and extraction of polyphenols by using a mixture of acetone and water. Antioxidant activity was assessed by using the following...
three test systems: TRAP (Total Radical-trapping Antioxidant Parameter), FRAP (Fer-ric Reducing Ability of Plasma/Ferric Reducing Antioxidant Power), and PCL (photo-chemiluminescence). All assays were used as recently described by Schlesier et al. 2. Briefly, in the TRAP assay the delay of the decrease of fluorescence of R-phycoerythrin was measured, while in the FRAP assay the formation of a coloured ferrous complex was determined. In the PCL test system, the delay of the formation of chemiluminescence was analyzed.

Statistical analysis: All results are given as mean values ± standard deviation. Differences between variables were tested for significance by using the general linear model (GLM) for the 2-way ANOVA procedure and the t-test (SPSS for Windows®, Release 10.07 (June 2000, SPSS Inc., Chicago)), using a level of significance of p<0.05. The results were defined as “comparable” if p >0.05.

Results and Discussion

Concentration of the major antioxidant in blood, uric acid, in plasma and serum was 0.30±0.06 mmol/L and did not change significantly (p>0.05) in plasma as well as in serum after ingestion of the beverage. Total phenolics content (GAE) as parameter of absorption of poly-phenols ranged from 0.68±0.34 to 0.84 ± 0.06 mmol/L in plasma and from 0.88±0.02 to 0.94±0.05 mmol/L in serum and was not significantly affected by the ingestion of the beverage.

The baseline values of antioxidant capacity for plasma and serum are shown in Table 2 for the three test systems used. Within plasma as well as within serum the three test systems resulted in significantly different antioxidant activities. In addition, the results of plasma and serum were significantly different within each assay, being lower in serum compared to plasma with TRAP and FRAP. With the TRAP assay, a significant increase was found only in serum 60 min after ingestion of the beverage (Fig. 1B), while in plasma (Fig. 1A) the antioxidant capacity did not change. The FRAP assay resulted in significantly increased antioxidant capacity in plasma (Fig. 2A) as well as in serum (Fig. 2B) very soon (30 min) after drinking the beverage rich in polyphenols. The PCL assay, in other inves-tiga-tions the most sensitive test system², did not show any alteration in anti-oxidant capacity neither in plasma nor in serum.

Data in the literature on antioxidant effects after ingestion of beverages rich in polyphenols are not consistent. Red wine and tea are the mostly used beverages in this context. Besides human studies with chronic ingestion of these beverages for 2-4 weeks, investigations with single doses are scarce. However, single dose experiments make it possible to estimate how fast the polyphenols are absorbed by the human organism.

In a study, where 13 volunteers (10 m, 3 f) ingested 550 mL...
red wine (low alcohol content 3.5%) daily in the evening for four weeks, Cu\textsuperscript{2+}-mediated LDL oxidation was not affected by this intervention and uric acid, vitamin C and glutathione in plasma as well as vitamin E in LDL particles were unchanged, too\textsuperscript{14}. In another study, where 20 volunteers (m) ingested 2 g alcohol-free red wine phenolics mixture (comparable to approximately 1 L red wine) daily for two weeks, antioxidant capacity as determined by chemiluminescence was significantly increased from 156.4±10.4 to 170.0±10.4 µmol/L Trolox equivalents, Cu\textsuperscript{2+}-mediated LDL oxidation was unchanged and a significant increase of vitamin E in LDL particles was observed\textsuperscript{5}. A single dose (10 volunteers, 4 m, 6 f) of 113 mL alcohol-free red wine (comparable to 300 mL non-dealcoholated red wine) with 3636±48 mg/L quercetin equivalents led to significantly increased TRAP values and contents of total phenolics 50 min after ingestion of the beverage but vitamin C, vitamin E, uric acid, and carotenoids in plasma were not affected\textsuperscript{13}. Single doses of 100, 200 and 300 mL red wine resulted in another human study (5 volunteers, m) in significantly increased TRAP values (+ 20-25%) 60 min after the intervention, but only after ingestion of 200 or 300 mL wine\textsuperscript{15}. Green tea extract (3 g/d, 4 w, 10 volunteers (f)), equivalent to 10 cups of tea, led to significantly decreased malondialdehyde (4.87±0.85 vs. 6.21±0.92 mmol/L) after four weeks of intervention but vitamin C, vitamin E, and glutathione were not affected by this intervention, indicating no antioxidant sparing effect\textsuperscript{16}. When 22 volunteers (m) ingested 750 mL black

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<th>Table 1. Main characteristics of the participants.</th>
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BMI: body mass index

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<th>Table 2. Baseline antioxidant capacity in plasma and serum as determined by using the FRAP assay, and the PCL assay.</th>
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\textsuperscript{a} Values within rows with different superscript letters are significantly different, p < 0.05

\textsuperscript{A-B} Values within columns with different superscript letters are significantly different, p < 0.05

\textsuperscript{A} 0 20 40 60 80 100 120 140

\textsuperscript{B} 0 20 40 60 80 100 120 140

Figure 2. Ferric reducing antioxidant power (FRAP) as parameter of antioxidant capacity in plasma (A) and serum (B) after ingestion of 500 mL of a beverage rich in antioxidants.
Simultaneously decreased levels of less active polyphenols of polyphenols out of the beverage including their metabolism (FRAP, TRAP), reaching the peak after different times. Ingestion within the polyphenolic pattern. In contrast, the antioxidant in plasma nor in serum. However, there might be differences polyphenols comprised in a beverage based on fruit juices did

Concluding our investigations, a single dose of plant the same three test systems (FRAP, ORAC, TEAC) were investigated on their antioxidant potential by using the FRAP, ORAC, and the TEAC assay. The responses serum and/or plasma investigations on their antioxidant potential affected by the intervention. Rice-Evans one test system to determine how antioxidant capacity was

Additionally, only scarce papers are available using more than one test system to determine how antioxidant capacity was affected by the intervention. Rice-Evans described results of serum and/or plasma investigations on their antioxidant potential by using the FRAP, ORAC, and the TEAC assay. The responses of uric acid, ascorbic acid, and α-tocopherol were comparable for all assays. TEAC values were similar for plasma and serum. In contrast, the FRAP values were significantly higher in plasma compared to serum (700 ± 183 µmol/L) compared to serum. ORAC values were significantly higher compared to all other results. In another study, serum samples of 45 volunteers (14 m, 31 f) were ingested by 6 volunteers (3 m, 3 f) as single dose and plasma was withdrawn before intervention and 1, 2, 4 and 6 hours after drinking the juice, a significant 30% increase was observed for the TEAC value 2 h after intervention in combination with a significant 18% decrease in malondialdehyde 4 h after intervention.

Human plasma is the most used physiological material for assessment of antioxidant capacity within intervention studies. In addition, only scarce papers are available using more than one test system to determine how antioxidant capacity was affected by the intervention. Rice-Evans described results of serum and/or plasma investigations on their antioxidant potential by using the FRAP, ORAC, and the TEAC assay. The responses of uric acid, ascorbic acid, and α-tocopherol were comparable for all assays. TEAC values were similar for plasma and serum. In contrast, the FRAP values were significantly higher in plasma compared to serum, being both lower than the TEAC values. ORAC values were significantly higher compared to all other results. In another study, serum samples of 45 volunteers (14 m, 31 f) were ingested on their antioxidant potential by using the same three test systems (FRAP, ORAC, TEAC). The results ranged as follows: ORAC > TEAC > FRAP. A sig-nificant correlation was observed between ORAC and FRAP, showing no correlation of both assays with the TEAC assay. Significant higher TRAP values in plasma (1480 ± 183 µmol/L) compared to serum (700 ± 391 µmol/L) were found for five smokers in another trial.

Conclusions

Concluding our investigations, a single dose of plant polyphenols comprised in a bev-erage based on fruit juices did not significantly increase the content of total phenolics neither in plasma nor in serum. However, there might be differences within the polyphenolic pattern. In contrast, the antioxidant capacity significantly rose in plasma (FRAP) as well as in serum (FRAP, TRAP), reaching the peak after different times. Ingestion of polyphenols out of the beverage including their metabolism might have enhanced the level of more active antioxidants. Simultaneously decreased levels of less active polyphenols might be the reason for the unchanged contents of total phenolics together with a significantly enhanced antioxidant poten-tial within plasma and serum. Clotting of the blood sample at room temperature for 30 min to obtain serum samples may lead to oxidation of phenolic compounds, changing their antioxidant behaviour. The third method used to determine the antioxidant activity, the photo-chemiluminescence test system, did not show any alteration in the antioxidant capacity nor in plasma nor in serum. Thus, the kind of physiological liquid as well as the kind of test system seem to be fac-tors affecting the analysis of antioxidant capacity within human inter-vention studies. These interactions will be further investigated. The use of at least two assays is strongly recom-mended. At the moment, only a ranking or a kinetic evaluation within each assay is possible.

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References


