ferrocene labels are brought into sufficient proximity to the electrode surface for detection. Application of an alternating current voltage to the electrode produces reversible reduction and oxidation of ferrocenes. Electrons are transferred between the label and the electrode surface only when the target is present and hybridized by both signaling probe and capture probe. The current generated by this system is detectable with the electronic detection system called the eSensor 4800 system, which can analyze 48 chips at a time. A molecular representation of the chemical structures on the electrode surface has been published (2).

The system has been described in more detail elsewhere (2, 3), and eSensor chips for clinical applications as well as detection of pharmacogenomic targets, infectious disease agents, genetic mutations, and industrial targets (transgenic crops, veterinary pathogens, and food safety) are in development. Chip density will range from 16 to 36 electrodes, providing a clinically relevant panel size.

References

Daniel H. Farkas
Clinical Diagnostics
Motorola Life Sciences
757 South Raymond Ave.
Pasadena, CA 91105
Fax 626-584-1471
E-mail dan.farkas@motorola.com

Is Sulfite an Antiatherogenic Compound in Wine?

To the Editor:
High intake of fats is strongly correlated with high mortality from coronary heart disease. The lack of this correlation not seen in certain regions of France has been attributed to wine consumption (1). Red wine contains phenolic substances. The antioxidant properties of these compounds may delay the onset of atherogenesis by reducing peroxidative reactions. Sodium sulfite is used as a preservative in foods and as an antioxidant in alcoholic beverages. Sulfite is present in finished wines in concentrations up to ~6 mmol/L. We measured the concentration of plasma sulfite in four healthy volunteers (males; age range, 28–37 years) before and after they drank a glass of wine (200 mL of a 1997 Bordeaux red wine containing ~320 mg/L sodium sulfite). Each volunteer fasted for 12 h before the study. Plasma sulfite was determined by a sensitive HPLC method described by Ji et al. (2).

Basal concentrations of sulfite were 0.43–1.20 μmol/L (mean ± SD, 0.72 ± 0.05 μmol/L). There was a sharp increase in plasma sulfite that reached maximum concentrations (5.8–9.2 μmol/L) in each individual at 0.5–1.0 h after the consumption of wine (Fig. 1). Thereafter, sulfite concentrations gradually decreased, but were higher at 4 h than basal values (paired t-test, P <0.01).

We examined the in vitro capacity of sulfite to prevent plasma lipid peroxidation. Human plasma was oxidized by incubation with 50 mmol/L 2,2’-azobis-(2-aminopropane) hydrochloride (AAPH) at 37 °C for 6 h with continuous shaking under air. This procedure generates alkyl peroxyl radicals at a constant rate (3). We then evaluated the formation of lipid peroxides as N-methyl-2-phenylindole-reactive substance, using the Bioxytech® LPO-586 method (Oxis International) and expressed as malondialdehyde equivalents. Sodium sulfite was added at concentrations of 10 and 100 μmol/L to the samples before incubation with AAPH. Sulfite suppressed the formation of lipid peroxides in a dose-dependent manner (lipid peroxide concentrations: control, 3.78 ± 0.05 μmol/L; 10 μmol/L sodium sulfite, 2.51 ± 0.85 μmol/L; 100 μmol/L sodium sulfite, 2.01 ± 0.62 μmol/L; ANOVA, P <0.01). In parallel, we examined the antioxidant activity of 100 μmol/L quercetin (lipid peroxide concentration: 2.11 ± 0.40 μmol/L; P <0.01), a phenolic compound in red wine that may account for the “French paradox” and thus is the subject of great research interest (4). Sulfite is as strong as quercetin in preventing plasma lipid peroxidation.

Finally we examined the antioxidant activity of orally administered sulfite. Three healthy volunteers drank 80 mg of sodium sulfite in water. Plasma samples from the volunteers were assessed for lipid peroxidation by exposure to 50 mmol/L AAPH. Formation of malondialdehyde was significantly lower in the plasma obtained after sulfite loading in each individual (before loading, 4.4 ± 0.61 μmol/L; after loading, 3.76 ± 0.62 μmol/L; paired t-test, P <0.01). Plasma sulfite concentrations were 6.4–10.2 μmol/L at this time point, which were similar to those seen in the volunteers who consumed a glass of wine. Thus, orally administered sulfite is capable of suppressing oxidative stress in plasma.

Several antioxidants have been shown to prevent the progression of atherosclerosis. Red wine contains higher amounts of phenolics than white wine, which may partly explain the French paradox. However, there is little information regarding the absorption of orally administered phenolics; this is partly a result of the lack of reliable assay systems for these compounds in the plasma.

We showed here an increase in
plasma sulfite, after the consumption of a glass of wine, to a concentration that may account for increased plasma antioxidant activity. We believe that these data justify further study of the role of sulfite as an antioxidant and antiatherogenic agent. Several important questions should be addressed, including whether there is any correlation between plasma sulfite concentrations and wine consumption or development of cardiovascular events in a large cohort.

References

Hideki Mitsuhashi
Hidekazu Ikeuchi
Yoshihisa Nojima*

Third Department of Internal Medicine
Gunma University
School of Medicine
Maebashi
Gunma, 371-8511 Japan

*Author for correspondence. E-mail ynojima@med.gunma-u.ac.jp.

Real-Time PCR Assay with Fluorescent Hybridization Probes for Rapid Interleukin-6 Promoter (−174G→C) Genotyping

To the Editor:
Interleukin-6 (IL-6) is a central protein in the regulation of the inflammatory and immunologic response (1). A G→C polymorphism at position −174 has been associated with osteoporosis (2), juvenile rheumatoid arthritis (3), and atherosclerosis (4) with increased risks in the absence of the CC genotype.

Restriction fragment length polymorphism analysis has been used for IL-6 genotyping, but it is time-consuming and requires multiple manual steps. To improve throughput, we developed a rapid-cycle PCR method (Roche Diagnostics) with fluorescent probe melting analysis. This assay was completed in 60 min.

DNA from 115 German Caucasians was extracted from whole blood according to standard procedures. Our study population consisted of 40 healthy blood donors and 75 patients from the intensive care unit of our hospital. The reliability of the proposed assay was confirmed by restriction enzyme digestion with SfaI. PCR was performed in disposable capillaries (Roche Diagnostics) with a reaction volume of 20 μL containing 2 μL of DNA (20–80 ng), 0.5 μM each of the primers (sense, 5′-TTA CTC TTT GTT AAG ACA TGC CA-3′; anti-sense, 5′-ATG AGC CTC AGA CAT CTC CAG-3′), 2 μL of reaction buffer [LightCycler fast start DNA master hybridization probes 10× buffer (1× = 1.75 mM); Roche Diagnostics], 1 μL of MgCl₂ (final concentration, 2.25 mM) and 0.2 μM each of the labeled probes. The anchor probe (5′-CTA AGC TGC ACT TTT TTC CCA AGT-3′) was labeled at the 3′ end with fluorescein. The sensor probe, which is specific for the G allele (5′-GTT TCT TGC GAT GCT AAA GGA-3′), was labeled with LightCycler Red 640 at the 5′ end and modified at the 3′ end by phosphorylation to block extension. The PCR conditions were as follows: initial denaturation at 95 °C for 10 min, followed by 45 cycles of denaturation (95 °C for 10 s, 20 °C/s), annealing (53 °C for 10 s), and extension (72 °C for 10 s). The melting curve consisted of one cycle at 95 °C for 0 s and 45 °C for 90 s, and then increasing the temperature to 85 °C at a rate of 0.1 °C/s.

The fluorescence signal (F2) was monitored continuously during the temperature ramp and then plotted against the temperature (T). These curves were transformed to derivative melting curves [−d(F2)/dT vs T].

Representative results for the three different genotypes (GG, GC, and CC) are shown in Fig. 1. Of the 115 samples tested, 33% were GG, 46% were GC, and 21% were CC. In a larger study (383 Caucasians), the most frequent genotype was GC, followed by GG and CC (3). In a direct method comparison, our proposed new technique and the restriction enzyme technique with SfaI gave identical genotyping results (data not shown). We conclude that the assay is rapid and accurate and seems especially suited for laboratories that process large numbers of samples.

We thank Andreas Nitsche and Oliver Landt (TIB MOLBIOL, Berlin, Germany) for designing the hybridization probes and reading the manuscript.

References
2. Ferrari SL, Garnero P, Emond S, Montgomery H, Humphries SE, Greenspan SL. A functional poly-