detected by adding 100 μL of anti-DNA monoclonal antibody (MAb 27–14-D9; Sorin Biomedica), which recognizes double-stranded DNA (dsDNA); after a 1-h incubation at 37 °C, we added to this 100 μL of horseradish peroxidase-labeled rabbit anti-mouse immunoglobulin (Sorin Biomedica) diluted 1:20 000 in phosphate buffer containing 10 mL/L fetal calf serum and incubated the mixture for another hour at room temperature. Single reactions were detected by adding 100 μL of “ETI-EIMA” chromogen/substrate mixture (27 g/L tetramethylbenzidine and 0.1 mmol/L hydrogen peroxide) and incubating in the dark for 30 min at room temperature. After stopping the reaction with 200 μL of 1 mmol/L sulfuric acid, we determined the genotype of each amplified sample by examining the pattern of colored wells or the absorbance at 450 nm.

All affected patients, who were homozygous for SMN deletion, were unambiguously detected by color development only in the well containing the CEN SMN-specific probe, whereas samples from unaffected individuals expressed color development in both wells (Fig. 1). Deviations of 2 °C from the selected temperature resulted in nonspecific hybridization. In our hands, the absorbance at 450 nm ranged between 1.01 and 2.0 after specific hybridization with both probes. The specificity of this method was assessed by comparing the results with those obtained by SSCP analysis [7] and by loading unrelated PCR products of noncomplementary sequences [cytosolic fibroblast transmembrane regulator (CFTR) exons 10–11, and human papillomavirus (HPV)] onto microtiter plates bearing SNM probes. The DEIA results unambiguously matched SSCP genotyping, and signals obtained with noncomplementary sequences were indistinguishable from background. The cutoff value, based on calculation of the mean for the negative control (0.125) + 3SD, was 0.320 ± 0.04 (mean ± SD; range 0.316–0.324). There were no false-positive results in these experiments at the selected cutoff values.

In conclusion, we have developed a PCR-based method that provides definitive data and is simple, sensitive, and reliable enough to be used in a routine screening. The use of an anti-dsDNA monoclonal antibody resolves the difficulty of DNA labeling. In fact, the hybridization itself generates the epitope recognized by the antibody, which represents the only signal source. This makes the DEIA much simpler than other ELISA-coupled assays, such as OLA (oligonucleotide ligation assay), which require the presence of fluorochrome-labeled probes. Besides, the procedure we report is faster and less expensive than electrophoresis-based deletion analysis. Hundreds of samples can be analyzed in 1 day by utilizing 96-well microtiter plates, a general assay scheme, and the reagents routinely used in an enzyme immunoassay.

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References


It is well established that hypercholesterolemia is an important cause of coronary heart disease (CHD), and clinical intervention studies have confirmed the effectiveness of therapy to aid patients with this condition. The Helsinki Heart Study (HHS) has demonstrated the effectiveness of lipid-lowering therapy for the primary prevention of CHD by reducing the total and low-density lipoprotein (LDL) cholesterol [1]. Over a 5-year period of follow-up of those entered in the HHS, cardiovascular events were reduced by 34% in the gemfibrozil-treated group. Post hoc analysis of the HHS findings indicates that an increase of high-density lipoprotein cholesterol is also thought to be the strongest indication of a possible reduction in future cardiovascular events. On the other hand, Fiji and Sobel reported that beneficial effects of gemfibrozil in reducing coronary events may depend, in part, on potentiation of fibrinolysis by direct diminution in synthesis of endogenous plasminogen activator inhibitor type 1 [2].

Previously, some have speculated that qualitative LDL changes, particularly the oxidation of LDL, are the most important factors in the formation of foam cells [3]. The most compelling evidence of the involvement of oxidized lipoproteins
in atherogenesis is the fact that atherosclerosis has been markedly ameliorated in experimental animals by treatment with antioxidants, e.g., probucol, which also inhibits LDL oxidation. M1, a metabolite of gemfibrozil that is hydroxylated on a phenol ring, i.e., 5-(4-hydroxy-2,5-dimethylphenox)-2,2-dimethyl pentanoic acid, may also have antioxidant properties, as we reported previously for structurally similar compounds [4].

We therefore initiated this preliminary study to determine whether M1 can inhibit in vitro LDL oxidation mediated by cupric ion or 2,2′-azobis(4-methoxy-2,4-dimethylvaleronitrile) (V-70). The vehicle in this test system was 100 mL/L ethanol solution, which has no inhibitory effects on LDL oxidation [4]. The results (Fig. 1) show that M1 does indeed have an in vitro antioxidative effect on copper-mediated LDL oxidation in a concentration-dependent manner, although its antioxidative effect is milder than that of probucol. The antioxidative effect of M1 on V-70-mediated LDL oxidation is similar (data not shown). Because we selected the concentrations of M1 used in these experiments to reflect the M1 concentrations in human plasma after gemfibrozil treatment, we think that part of the success rate achieved in reducing cardiovascular events among patients in the HHS might also be due to the direct antioxidative effect of M1.

![Graph](image)

Fig. 1. Dose response of inhibition of gemfibrozil metabolite on conjugated diene formation during copper-mediated LDL oxidation. All of the reactions represented above also contained Cu²⁺, 5 μmol/L.

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Measurement of prostate-specific antigen (PSA) is used for monitoring and, more recently, to help detect prostate cancer [1, 2]. Much of the published clinical research demonstrating the role of PSA was conducted with the Tandem PSA assay, and the medically accepted normal cutoff for PSA of 4 μg/L was derived through the use of this assay [3–5]. Initially, and then during development of most of the recently available immunoassays for PSA, the fact that various forms of PSA were circulating was unappreciated [6].

At least two forms of PSA, free PSA (f-PSA) and PSA complexed to α₁-antichymotrypsin (PSA-ACT), are detected by commercial PSA assays [7], and researchers are now describing different reactivities of those forms in different commercial PSA assays. Brawer et al. [8] demonstrated systematic differences between the IMx® and Tandem PSA assays and concluded that both calibration and PSA forms were the source of those differences. Jacobsen et al. [9] compared the responses of IMx and Tandem assays to normal sera and showed that, for lower free-to-total PSA (f-PSA/t-PSA) ratios, the IMx assay produced lower responses than Tandem and, for higher f-PSA/t-PSA ratios, the IMx assay produced higher responses than Tandem.

In this study we compared the IMx®-E PSA (Hybritech, San Diego, CA), and IMx®-PSA (Abbott Labs., Abbott Park, IL) assays for PSA in the clinically relevant range for detecting prostate cancer, 2–20 μg/L, and examined the effect of the %f-PSA ([f-PSA/t-PSA] × 100) in each serum sample on the discordance between the two methods.

Serum samples with 2–20 μg/L PSA (by Tandem-E PSA) were selected without conscious bias from an in-house bank. Experiment 1 assayed 62 specimens; experiment 2, the follow-up study, was conducted with 102 different specimens. All assays within each experiment were run on the same day. IMx and Tandem PSA reagent lots were obtained from commercial stock available at the time of the experiments; different lots were used for each experiment. All serum samples were tested in duplicate. Tandem-E and f-PSA data were generated at Hybritech and IMx data were generated at a reference laboratory. The f-PSA assay is described elsewhere [10].

In experiment 1 (n = 62), regression analysis of the t-PSA results for IMx (y) vs Tandem (x) gave a slope of 0.91, an intercept of 0.12, and r² = 0.91. In experiment 2 (n = 102), a similar regression analysis of t-PSA results for IMx (y) vs Tandem (x) gave a slope of 0.71, an intercept of 1.15, and r² = 0.77. The mean ratios of IMx/Tandem values for the same samples were 0.93 and 0.82 for experiments 1 and 2, respectively.

Figure 1 shows results from both experiments, expressed as a ratio of IMx/Tandem vs the ratio of f-PSA/t-PSA in the samples. As the f-PSA/t-PSA ratio increases, so does the IMx/Tandem ratio (slope = 1.34, y-intercept = 0.71, r = 0.478). The observed slope is statistically different from zero with P < 0.0001.

The regression analyses of t-PSA in experiments 1 and 2 show a variable and therefore problematic relationship between the

References


The rega...