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The measurement of the lactonase activity of paraoxonase-1 in the clinical evaluation of patients with chronic liver impairment

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Abstract

Aims: We investigated the analytical performance of a new assay of the lactonase activity of paraoxonase-1 and its efficacy in the assessment of liver damage.

Design and methods: Serum lactonase activity was determined by the hydrolysis of 5-thiobutyl butyrolactone in 633 healthy individuals and 369 patients with chronic liver disease. Paraoxonase-1, 2, and 3 gene polymorphisms were analyzed by the MassArray™ method.

Results: Linearity was up to 10 U/L. Detection limit was 0.12 U/L. Imprecision was ≤17.7%. Lactonase values in our normal population were 5.99 (3.29–13.61) U/L. Lactonase activity showed a lower influence of genetic polymorphisms than the classical assay using paraoxon. Both measurements showed a similar efficiency in testing for liver dysfunction.

Conclusion: We report a reliable assay using a non-toxic substrate for the measurement of serum lactonase activity. The influence of genetic variability is low. The assay could be a useful addition to tests evaluating liver impairment.

Keywords: Diagnostic accuracy; Lactonase; Liver diseases; Paraoxonase; Reference interval

Introduction

Paraoxonases are a family of three enzymes termed PON1, PON2 and PON3, whose genes are located adjacent to each other on chromosome 7q21–22 [1,2]. All these enzymes have had polymorphisms identified in the coding as well as the promoter regions, and their impact on their respective protein products is the subject of ongoing research [3]. The best known among the paraoxonases is PON1 which acts as an esterase and lactonase catalyzing the hydrolysis of organophosphates and other xenobiotics. It is found in the circulation bound to high-density lipoproteins (HDL) [4–6]. The original function of PON1 was that of a lactonase, and lipophylic lactones constitute its primary substrates [5,7]. PON1 degrades oxidized phospholipids in low-density lipoproteins (LDL) and HDL and, as such, plays a role in the organism's antioxidant system [8–11]. Alterations in circulating PON1 levels are associated with a variety of diseases involving oxidative stress [12–14].

The liver plays a key role in the synthesis of PON1 [15,16] and chronic liver diseases are associated with increased oxidative stress and lipid peroxidation [17,18]. We have reported [19–21] that serum PON1 activity is decreased in patients with chronic liver impairment, and have suggested that serum PON1 activity measurement may improve the evaluation of liver function in these patients. The potential clinical interest of these observations is high but there are some major

Abbreviations: DTNB, 5,5′-dithio-bis-2-nitrobenzoic acid; HDL, high-density lipoproteins; LDL, low-density lipoproteins; PON, paraoxonase; TBBL, 5-thiobutyl butyrolactone.

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limitations in the current methods for PON1 measurement that restrict its applicability. PON1 activity is usually determined by measuring the hydrolysis of paraoxon, a highly toxic and unstable compound which is not suitable for a high-throughput routine method. Secondly, the interpretation of the physiological significance of a measurement conducted with such an unnatural substrate is questionable. In addition, the enzyme's activity on paraoxon is strongly influenced by the $PON1_{192}$ genetic polymorphism [4]. For these reasons, substantial improvement in the choice of substrate is mandatory before applying this analysis in the clinical setting.

Recently, a new fast, semi-automated PON1 assay has been reported. The method is based on a chromogenic lactone that resembles the proposed natural lipolactone substrates. Preliminary data suggest that this method is reliable in measuring HDL-associated PON1 [22]. The present study was aimed at: *(a)* evaluating the analytical performance of a new lactonase activity paraoxonase-1 assay; *(b)* investigating the influence of genetic variability in a population-based study; *(c)* investigating the efficacy of the lactonase assay in the assessment of liver damage.

Materials and methods

Study participants

We analyzed samples from a population-based study conducted in our area. Details of this study have been published [23]. The participants were healthy subjects ($n=633$; 339 women, 294 men; mean age: 45 years, range 18 to 81) of Caucasian ethnic origin from the Mediterranean region of Catalunya. All the volunteers had been invited to attend a clinical examination and to provide a fasting blood sample. There was no clinical or analytical evidence of renal insufficiency, liver damage, neoplasia or neurological disorders.

The patient population consisted of 369 individuals with chronic liver disease (266 men and 103 women; mean age: 47 years, range 20 to 80 years) who had been admitted to one of the participating hospitals for treatment. The etiology of the disease was alcoholic in 178 (48%) and viral in 191 (52%) individuals. Samples for analyses were obtained within 24 h of admission. For the purpose of the present study, patients were segregated into two groups. One group ($n=215$; 158 men and 57 women) contained those patients with mild-to-moderate liver disease with mild hepatomegaly and/or a moderate increase in serum aminotransferase or γ-glutamyl transferase activities, but with no ultrasound changes that would suggest cirrhosis. The second group ($n=154$; 108 men and 46 women) contained those patients with liver cirrhosis confirmed by histology examination. The study was approved by the Ethics Committees of the Hospitals involved in the study and all patients and control subjects gave written consent to participation.

Biochemical measurements

Blood samples were collected after an overnight fast into tubes with no anticoagulants to obtain serum, or into tubes with K_2-EDTA for genetic analyses, or into Na_2-citrate tubes for plasma prothrombin time measurements. The analyses were performed immediately, or aliquots of material were stored at −80 °C for subsequent batched analyses. Serum lactonase activity was analyzed by measuring the hydrolysis of 5-thiobutyl butyrolactone (TBBL) as described [22]. All assays were performed on 96-well plates (Nunc™, Roskilde, Denmark) using an automated microplate reader (BioTek Instruments Inc., Winooski, VT, USA). Lactonase activity was measured in an assay reagent containing 1 mmol/L $CaCl_2$, 0.25 mmol/L TBBL and 0.5 mmol/L 5,5′-dithio-bis-2-nitrobenzoic acid (DTNB) in 0.05 mmol/L Tris–HCl buffer (pH = 8.0). The change in absorbance was monitored at 412 nm. Activities were expressed as U/L (1 U = 1 mmol of TBBL hydrolyzed per minute). The shelf-life of TBBL solution is approximately 2 months and stocks were replenished within this period. Serum paraoxonase activity was determined by measuring the rate of hydrolysis of paraoxon at 410 nm and 37 °C in a 0.05 mmol/L glycine buffer (pH = 10.5) with 1 mmol/L $CaCl_2$ [19]. Activities were expressed as U/L (1 U = 1 μmol of paraoxon hydrolyzed per minute). Paraoxon substrate had to be prepared fresh for every batch of measurements. Serum alanine aminotransferase (ALT), and γ-glutamyl transferase (GGT) activities and cholesterol, triglycerides, albumin, bilirubin, apolipoprotein A–I, and HDL–cholesterol concentrations were measured with reagents obtained from Beckman-Coulter in a Synchron Lxi automated analyzer (Beckman-Coulter, Fullerton, CA, USA). Plasma prothrombin time was measured in an ACL 9000 analyzer (Instrumentation Laboratories, Milan, Italy).

Control materials

There are no commercial control materials for use in performance studies of lactonase activity measurement. Instead, we used three pools of sera prepared from samples from the healthy population described above. Sera with the lowest, the highest, and the intermediate lactonase activities were pooled, gently mixed for 2 h at 4 °C and divided into aliquots for storage at −40 °C. The three pools were "Pool A" (3.96 ± 0.70 U/L), "Pool B" (5.36 ± 0.70 U/L), and "Pool C" (8.00 ± 0.94 U/L).

PON1, PON2, and PON3 genotyping

Genomic DNA was obtained from leukocytes (Puregene DNA Isolation reagent set, Gentra Systems Inc., Minneapolis, MN, USA). Selected single nucleotide polymorphisms (SNP's) were analyzed by the Iplex Gold MassArray™ method (Sequenom Inc., San Diego, CA, USA) at the Spanish National Genotyping Center (*Centro Nacional de Genotipado, Universitat Pompeu Fabra*, Barcelona, Spain).

Statistical analysis

Differences between groups were assessed with the Student's *t*-test (parametric) or the Mann–Whitney *U* test (non-parametric). Pearson or Spearman correlation coefficients were