Serum Paraoxonase Levels In Patients with Acute Liver Disease

C. M. Bindu · Usha Anand · C. V. Anand

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Abstract Paraoxonase is an anti-oxidant enzyme, which circulates in the plasma, tightly bound to HDL. This enzyme is known to be synthesized in the liver. This study was carried out in order to ascertain the diagnostic utility of this enzyme in acute liver disease. Serum basal as well as salt (NaCl) stimulated paraoxonase was estimated in 50 patients with an established diagnosis of acute liver disease and also in 50 healthy blood donors. Paraoxonase levels were significantly lower in patients as compared with controls ($P < 0.05$). The ‘receiver operating characteristic’ plot showed that this enzyme has a high degree of sensitivity and specificity for the diagnosis acute liver disease. Serum PON is likely to emerge as an additional test of liver function, as it encompasses three different attributes of hepatic function namely, synthetic capacity, detoxication and secretory functions.

Keywords Paraoxonase · Basal PON · Salt-stimulated PON · Liver function tests

Introduction

Paraoxonase (PON) is an aryldialkylesterase (EC 3.1.8.1) that is present in the plasma of mammals [1]. It was first discovered in the field of toxicology because of its ability to hydrolyze xenobiotics such as paraoxon and was

therefore given the name paraoxonase [2]. It circulates in plasma associated with high-density lipoprotein [3]. It has been shown to function as an anti-oxidant capable of protecting low density lipoproteins against peroxidative reactions [4]. Three different isoforms of PON have been described namely PON 1, PON 2 and PON 3 which constitute a family of enzymes [5].

The liver has a central role to play in the metabolism of lipoproteins [6]. PON1 is synthesized mainly in the hepatocytes [7]. It would therefore be relevant to study this enzyme in acute liver disease due to the following reasons. Liver disease could lead to quantitative and/or qualitative changes in plasma lipoproteins accompanied by changes in PON. Lipid peroxidative processes are known to occur in acute infective hepatitis while PON is anti-oxidative in nature [8]. PON is involved in detoxification of xenobiotics [2] and assessment of the ability of the liver to detoxify exogenous compounds has been used as a test of liver function.

It is a well established fact that PON is stimulated by calcium ions, an essential cofactor for the aryl esterase activity of the enzyme [9]. Apart from this PON activity is also stimulated by the presence of 1 M NaCl. It is customary to refer to the activity in presence of calcium ions alone as basal PON and that in the presence of both calcium ions as well as NaCl as salt-stimulated PON [10].

This study was carried with the following aims and objectives. Firstly, it was to be established whether acute liver disease produced any significant changes in serum PON activities. The second objective was to determine whether there was any correlation between serum PON and the various routine liver function tests. Finally the diagnostic efficacy of basal and salt-stimulated PON in assessing patients with acute liver disease was also to be determined using ‘receiver operating characteristic’ plot (ROC).

C. M. Bindu
Department of Biochemistry, Raja Rajeshwari Medical College,
Bangalore, India

U. Anand · C. V. Anand (✉)
Department of Biochemistry, PSG Institute of Medical Science
and Research, Peelamedu, Coimbatore 641004, Tamilnadu, India
e-mail: anand_vasudevarao@yahoo.com;
anandvasudevarao@gmail.com

In the present study, the enzyme that was estimated was taken to represent PON and not PON 1. This is because the method used for the assay, does not discriminate between the various forms of PON.

Materials and Methods

Subjects

There were two groups of subjects namely ‘cases’ and ‘controls’.

Cases

This group had patients with a diagnosis of acute liver disease, based on clinical examination and laboratory data. It consisted of 50 patients of either sex and age greater than 18 years.

Controls

This group had 50 healthy human adults who were voluntary blood donors of the hospital.

Informed consent was obtained from these subjects. The study was approved by the institutional human ethical committee.

Sampling

Blood samples (4 ml) were drawn from the subjects using vacutainers containing clot activators. The blood was allowed to clot, centrifuged and the serum was used to perform the biochemical analysis on the same day.

Biochemical Parameters

The liver function tests were performed on Dade Behring Dimension AR, a fully automated clinical chemistry analyzer. The LFT profile included serum total bilirubin (TBil), direct bilirubin (DBil), total protein (TP), albumin (Alb), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP).

Serum PON

This enzyme was estimated spectrophotometrically using 5.5 mM 4-nitrophenylacetate as the substrate in 20 mM Tris–HCl buffer at a pH of 8.0. The increase in absorbance due to the formation of the yellow 4-nitrophenol was monitored at 412 nm for 3 mins.

For each sample basal PON activity as well as salt-stimulated PON activity was determined as described below [11].

Basal PON: This was estimated by using the Tris–HCl buffer containing only 1 mM calcium chloride.

Salt-stimulated PON: This was estimated by using the same Tris–HCl buffer which however contained 1 mM calcium chloride as well as 1 M NaCl.

PON activity was calculated after corrections were made for non-enzymatic hydrolysis.

PON was taken to be 1 U/l when the rate of formation of the product 4-nitrophenol was 1 $\mu\text{mol/min}$ under the assay conditions.

Chemicals

4-nitrophenol acetate was procured from Sigma Chemical Company (St. Louis, MO, USA). All other chemicals used for the assay of PON were of analytical grade.

Statistical Analysis

The comparison between groups was done using the student's ‘*t*’ test. A *P* value <0.05 was considered to be statistically significant. The Pearson's correlation coefficient was also calculated between various parameters. The receiver operating characteristic (ROC) curves were plotted to test the diagnostic utility of PON as a test of liver function. Statistical analysis was done using ‘MedCalc’ software.

Results

The liver function tests of all subjects belonging to the ‘control’ group were well within the reference range. The LFT profile of the ‘cases’ group is shown in Table 1.

The basal PON as well as the salt-stimulated PON activities were significantly lower in acute liver disease as compared with healthy controls as shown in Table 2. The

Table 1 Routine LFT of ‘cases’

Test	Units	Reference range	Mean $\pm$ S.D.	Minimum	Maximum
T Bil	mg/dl	0.3–1.0	7.82 $\pm$ 6.29	2.05	28.14
D Bil	mg/dl	0.1–0.3	5.43 $\pm$ 5.09	0.34	23.94
TP	g/dl	6.4–8.3	6.38 $\pm$ 1.05	4.20	8.10
Alb	g/dl	3.4–4.8	2.95 $\pm$ 1.00	1.20	5.40
AST	U/l	10–40	278.7 $\pm$ 545.7	12	2588
ALT	U/l	10–35	289.6 $\pm$ 576.4	21	2434
ALP	U/l	35–130	277.6 $\pm$ 294	53	1480

Table 2 Basal and salt-stimulated PON in 'cases' and 'controls'

Groups <i>n</i> = 50	Basal PON ^a U/ml	Salt-Stimulated PON ^a U/ml
Cases (M/F = 42/8)	335.86 ± 138.93	426.91 ± 151.31
Controls (M/F = 45/5)	558.84 ± 43.71	649.98 ± 78.89
' <i>P</i> ' value control versus cases	<0.001	<0.001

^a Values represent Mean ± S.D

basal PON values ranged from 89.98 to 593.87 U/l with a median value of 349.08 U/l in the 'cases' group while in the 'control' group it ranged from 421.07 to 689.17 U/l with a median value of 556.04 U/l. The salt-stimulated PON ranged from 104.30 to 719.84 U/l with a median value of 413.8 U/l in the 'cases' group and from 509.21 to 843.97 U/l with a median value of 652.25 U/l.

The PON values of the two groups are depicted in the 'box and whisker' plots in the Graphs 1 and 2. These graphs show how the values are clustered together in the 'control' group as compared with the 'cases' group. The box has been constructed using the median value, the 25th

Graph 1 Box and whisker plot—serum basal PON. The boxes extend between the 25th and 75th percentile. The medians of the two groups are linked for comparison. The whiskers indicate the minimum and maximum value for the two groups

Graph 2 Box and whisker plot—serum salt stimulated PON. The boxes extend between the 25th and 75th percentile. The medians of the two groups are linked for comparison. The whiskers indicate the minimum and maximum value for the two groups

Fig. 1 Receiver operating characteristic curves. This graph shows that both basal (BPON) and salt-stimulated (SSPON) have a high specificity and sensitivity as a test of liver function. The AUC is 0.948 for basal PON and 0.883 for salt-stimulated PON

and 75th percentile. The box is wider for the 'cases' group when compared with the 'control' group for both basal as well as salt-stimulated PON.

The percentage of activation of PON by NaCl was only 16% while it was 27% for the liver disease group.

There was a significant positive correlation between basal PON and serum total and direct bilirubin ($P < 0.05$). There was a significant negative correlation between serum PON and serum total protein, albumin and ALT ($P < 0.05$).

The ROC plot was used to compare the efficacy of the two forms of the enzyme in assessing liver function. This was done by making use of all the 100 subjects and classifying them on the basis of presence or absence of liver disease. The ROC plot showed that the sensitivity and specificity of basal PON was significantly higher than that of salt-stimulated PON as a parameter to assess acute liver disease. The area under the curve (AUC) was 0.948 for basal PON, the 95% CI being 0.885–0.983. The AUC for salt-stimulated PON was 0.883, the 95% CI being 0.804–0.939. The '*P*' value for the ROC plot of basal as well as salt-stimulated PON was 0.0001. The pair-wise comparison of ROC curves showed a difference of 0.0648 with a '*P*' value of 0.014 (Fig. 1).

Discussion

A number of studies have estimated serum PON 1 in humans [8] and experimental animals [8] with chronic liver disease. They have observed that serum PON 1 levels

decline in various types of liver diseases. In contrast, there are only a few reports on this enzyme in acute liver disease [12]. Despite these reports; the present study was carried out because we wanted to ascertain PON activity using 4-nitrophenol acetate rather than paraoxon as the substrate. It is possible that the results obtained with one substrate might be quite different from that obtained with another. Paraoxon is known to be toxic and unstable and therefore cannot be introduced into routine practice, if and when this enzyme gets included in the panel of tests offered by clinical chemistry laboratories [13]. Also, in the present study, both basal PON as well as salt-stimulated PON have been estimated and it has been demonstrated that there is significant fall in both forms of the enzyme. The sensitivity and specificity of both basal PON as well as salt-stimulated PON was statistically significant. However, if a single determination needs to be done, basal PON should be the activity of choice as indicated by the higher 'area under the curve' value in the ROC plot.

It is an established fact that acute liver disease is accompanied by lipid peroxidative processes [14]. PON is an enzyme which contains a sulphhydryl group at the active site. Aviram and his co workers have shown that when this enzyme is pre-incubated with parahydroxy mercuric benzoate, a reagent which is known to inactivate enzymes containing SH group at the active site, the protective effect of the enzyme against LDL oxidation was lost [15]. The lowering of PON in acute liver disease could therefore be attributed to peroxidative changes that occur in the hepatocytes.

The results of the present study have shown that the percentage of activation in the presence of NaCl was 16% for control and 27% for patients. The reason for this wide difference in salt stimulation is not known with certainty. However, taking into consideration the possible inactivation of PON within the hepatocytes, the following explanation could be offered.

PON could be undergoing changes in physico-chemical properties as a result of exposure to free radicals; this in turn could lead to alterations in the substrate binding and catalysis [16]. Addition of NaCl in some way enhances the binding and hence catalysis. The difference between the mean basal activity and mean salt-stimulated activity becomes more pronounced in patients. The calculation of the percentage of activation, might further enhance the value of PON as part of LFT.

There are several tests which are routinely carried out to assess the functional capacity of the liver. Since the liver performs a wide range of functions, these tests serve to elucidate the derangement of a particular type of function; for example, the synthetic function is assessed by estimation of serum total proteins and serum albumin. Detoxification function is assessed by the hippuric acid synthesis

test while the secretory capacity is assessed by bromosulphthalein excretion test [17]. These tests have now become obsolete and currently there is no test being done to assess these two aspects of hepatic function. PON is an enzyme which is synthesized in the liver and secreted after being incorporated into HDL. Since it is an anti-oxidant, it has role in detoxification. A fall in serum PON could be taken as a manifestation of the derangement of all the above functions namely, synthesis, detoxication and secretion.

To conclude, this study has demonstrated that both basal as well as salt-stimulated PON decline in patients with acute liver disease. With the specificity and sensitivity of this enzyme being high, it could emerge as a parameter to assess liver function.

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