Iranian Journal of Pharmaceutical Research (2004) 3: 155-158
Received: December 2003
Accepted: May 2004

Original Article

Paraoxonase Inhibition by Propranolol

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Abstract

There are many evidences that human serum paraoxonase activity modifies plasma lipid profile and paraoxonase has an antiatherogenic property. Non-selective beta-blockers affect plasma lipid profile too, but they have atherogenic property when patients take these drugs in long term. In this study the effect of propranolol, a non-selective beta-blocker, on paraoxonase activity was investigated. Lineweaver-Burk and secondary plots were drawn and showed that propranolol is a mixed non-competitive inhibitor of paraoxonase.

Keywords: Paraoxonase; Non-selective beta-blockers; Propranolol.

Introduction

Human serum paraoxonase (PON1, EC 3.1.8.1) is a Ca^{2+} dependent, 45 kDa glycoprotein that is associated with high density lipoprotein (HDL). PON1 hydrolyses organophosphates, insecticides and nerve gases. Although PON1 can offer protection against the toxicity of some organophosphates, its physiological role is still not known. However, evidence exists for a protective effect of PON1 against oxidative damage. It retards the oxidation of low density lipoprotein (LDL), both in vivo and in vitro, by hydrolyzing the lipid peroxides formed in plasma (1-4). It has been suggested that PON1 is related to coronary heart disease risk. PON1 activity was reported to be lower in subjects with familial hypercholesterolemia, the disease that lead to the development of atherosclerosis. PON1 activity is under genetic and environmental regulation and appears to vary widely among individuals and populations (5).

Beta-blockers are widely used to treat cardiovascular diseases. It has been shown that non-selective beta-blockers affect the concentration and oxidizability of plasma lipids. They tend to increase triglycerides and LDL, while decreasing the atheroprotective HDL (10). There is no previous report on the effect of beta-blockers on PON1 activity. Propranolol is a well known non-selective beta-blocker widely used in the treatment of arrhythmia, angina and hypertension.

The aim of the present study was to investigate whether propranolol, a non-selective beta-blocker could affect PON1 activity.

Experimental

PON1 activity was measured spectrophotometrically. PON1 hydrolyses paraoxon (substrate) in the presence of Ca^{2+} and Na^{+} and as a result *p*-nitrophenol is liberated (11, 12). An Increase in absorbance at 412 nm is related to PON1 activity. No absorbance was observed in visible range for plasma itself.

Materials

Paraoxon was purchased from Sigma-Aldrich chemie GmbH (Germany).

Propranolol was obtained from Tolid Daru (Iran). Other chemical compounds were from

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Merck Co. (Germany). UV absorbance was measured with a SHIMADZU 160-A spectrophotometer. Serum was obtained from a fast, healthy, non-smoker, male volunteer.

Solutions

Solution A: glycine/NaOH buffer (50 mM, pH=10.0) containing 1.0 M NaCl and 1.0 mM CaCl2 was prepared.

Solutions B1-B6: Paraoxon was added to solution A to reach final concentrations of2.5, 5.0, 7.5, 10, 12.5 and 15.0 mM respectively.

Solutions C1-C6: Propranolol (as the base) was dissolved in methanol to prepare concentrations of 0.25, 0.5, 0.75, 1.0, 1.25 and 1.5 mM respectively.

Methods

PON1 activity measurement

Four hundred µl of a 1/5 prediluted serum sample with distilled water, was added to 4.1 ml solution A and then 500 µl of one of the solutions B_1-B_6 was added. The rate of paraoxon hydrolysis was assessed by measuring liberation of *p*-nitrophenol at 412 nm at 25 C (ε=17000, pH=10.0). For subtraction of non-enzymatic hydrolysis, blanks (samples without serum) were used. Enzyme activities were expressed in international units (U) per milliliter of serum. One U corresponds to the quantity of enzyme that hydrolyses 1 µmol of substrate per minute at the given pH and temperature.

Inhibition assay

1) Four hundred µl of a 1/5 prediluted serum sample with distilled water, was added to 4.0 ml solution A and then 100 µl one of the solutions C_1-C_6 was added. After 10 min incubation, 500 µl of solution B6 was added and the absorbance measured at 412 nm. 2) Four hundred µl of a 1/5 prediluted serum sample was added to 4.0 ml solution A followed by the addition of 100 µl of solution C_2. After 10 min incubation, 500 µl of one of the solutions B1-B6 was added and absorbance measured at 412 nm.

Results And Discussion

The enzyme kinetic (with and without propranolol) has been shown as the Lineweaver-Burk plot in figure 1. Figure 1 shows that propranolol is a mixed non-competitive inhibitor of PON1. Hence the equation describing the kinetics is a modified form of the Lineweaver-Burk equation:

$$\frac{1}{V} = \frac{Km}{Vm}\left(1+\frac{[I]}{Ki}\right).\frac{1}{[S]} + \frac{1}{Vm}\left(1+\frac{[I]}{\alpha Ki}\right)$$

Secondary plots were drawn and shown in figures 2-a and 2-b. PON1 kinetic parameters were calculated (Km=0.4 mM, Vm=0.255 $mol.min^{-1}.ml^{-1}$ serum) and inhibition parameters were obtained through Figures 2-a and 2-b (K_i=37 µm, αK_i=K_I=79 µM, α=2.13).

Based on the previous reports, PON1 activity is under genetic and environmental regulation. Regarding the environmental parameters, it has been reported that mice which had consumed red wine had less oxidized LDL, presumably related to an enhanced serum PON1 activity in these polyphenol-treated mice (6).

The inhibition of LDL oxidation by HDL is due to the hydrolysis of lipid peroxidases and the resulting inhibition of lipid peroxides appears to be, at least in part, a function of the enzyme paraoxonase, which is a component of HDL (2, 13). It has been shown that PON1 destroys the multioxygenated molecules found in oxidized phosphatidycholine. Furthermore, it has been demonstrated that inactivation of PON1 reduces the ability of HDL to inhibit

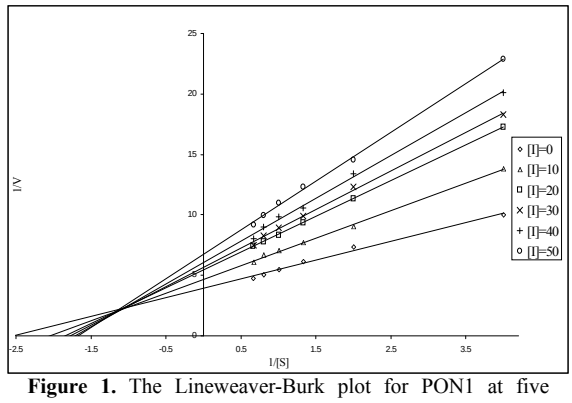

Figure 1. The Lineweaver-Burk plot for PON1 at five different concentrations of propranolol (I) in the presence of six different substrate concentrations. V values are expressed in $\mu mol.min^{-1}.ml^{-1}$ serum; [I] values have been expressed in µM. R-sq for [I] = 0, 10, 20, 30, 40, 50 are 0.981, 0.997, 0.999, 0.998, 0.996 and 0.998 respectively.

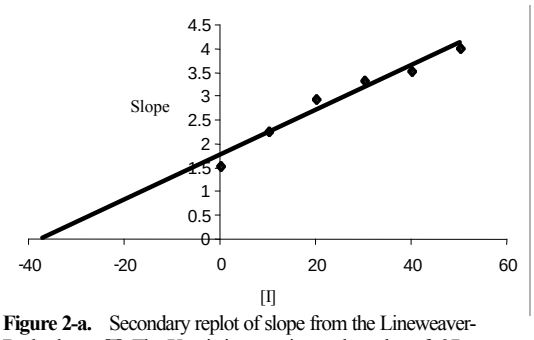

Figure 2-a. Secondary replot of slope from the Lineweaver-Burk plot vs [I]. The X-axis interception at the value of -37 represents the $-K_i$ parameter. [I] values are expressed in μM. R-sq is 0.978.

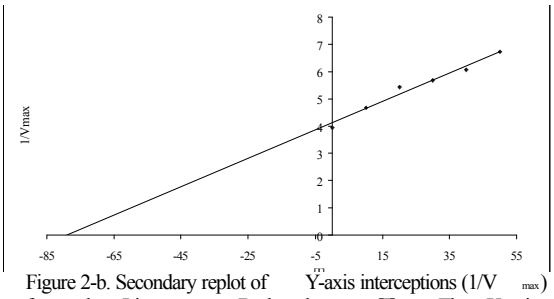

Figure 2-b. Secondary replot of Y-axis interceptions ($1/V_{max}$) from the Lineweaver -Burk plot vs [I]. The X-axis interception at the value of -79 represents $-K_i$ parameter . v values are expressed in μmol.min^{-1}.ml^{-1} serum. [I] values are expressed in μM. R-sq is 0.982.

LDL modification. It also reduces the ability of HDL to inhibit monocytic-endothelial interactions. They both appear to be important in the inflammatory response of arterial wall cells, which promotes atherogenesis (3). PON1 reduces mildly oxidized phospholipids by eliminating oxidized derivatives of unsaturated fatty acids (2, 3). It has also been shown that smoking is associated with a reduced serum PON1 activity and concentration (7). However, vitamins C and E intake are associated with an increased PON1 activity (8).

Serum PON1 activity is significantly increased during treatment with simvastatin (9). These evidences imply the importance of jointly considering environmental factors that modify PON1 activity.

Overall, in this study we found that propranolol, a well known non-selective beta-blocker, is a mixed non-competitive human serum paraoxonase inhibitor. Thus, it is possible that the use of propranolol could be influencing PON1 activity and its effect on the concentration and oxidizability of plasma lipids may be related to this property.

Acknowledgement

This work was partially supported by a grant from Tehran University of Medical Sciences Research council.

References

(1) Mackness MI, Arrol S and Durrington PN. Paraoxonase prevents accumulation of lipoperoxides in Low-density lipoproteins. *FEBS Letts.* (1991) 286: 152- 4

(2) Mackness MI, Arrol S, Abbott CA and Durrington PN. Protection of low-density lipoprotein against oxidative modification by high-density lipoprotein associated paraoxonase. *Atherosclerosis* (1993) 104: 129-35

(3) Watson AD, Berliner JA, Hama SY, La Du BN, Fault KF, Fogelman AM and Navab M. Protective effect of high density lipoprotein associated paraoxonase. Inhibition of the biological activity of minimally oxidized low density lipoprotein. *J. Clin. Invest.* (1995) 96: 2882-91

(4) Aviram M, Rosenblat M, Bisgaier CL, Newton RS, Primo-Parmo SL and La Du BN. Paraoxonase inhibites high-density lipoprotein oxidation and preserves its functions, a possible peroxidative role for paraoxonase. *J. Clin. Invest.* (1998) 101: 1581-90

(5) Mackness B, Mackness MI, Durrington PN, Arrol S, Evans AE, McMaster D, Ferriers J, Ruidavets JB, Williams NR and Howard AN. Paraoxonase acitivity in two healthy populations with differing rates of coronary heart disease. *Eur. J. Clin. Invest.* (2000) 30: 4-10

(6) Hayek T, Fuhrman B, Vaya J, Rosenblat M, Belinky P, Coleman R, Elis A and Aviram M. Reduced progression of atherosclerosis in apolipoprotein E-deficient mice following consumption of red wine, or its polyphenols quercetin or cathechin is associated with reduced susceptibility of LDL to oxidation and aggregation. *Arterioscler. Thromb. Vasc. Biol.* (1997) 17: 2744-52

(7) James RW, Leviev I and Righetti A. Smoking is associated with reduced serum paraoxanase activity and concentration in patients with coronary artery disease. *Circulation* (2000) 101: 2252-7

(8) Jarvik GP, Trevanian N, Mckinstry LA, Wani R, Brophy VH, Richter RJ, Schellenberg G D, Heagerty PJ, Hatsukami TS and Furlong CE. Vitamin C and E intake is associated with increased paraoxonase activity. *Arterioscler. Thromb. Vasc. Biol.* (2002) 22: 1329-33

(9) Tomas M, Senti M, Garcia-Faria F, Vila J, Torrents A, Covas M and Marrugat J. Effect of simvastatin therapy on paraoxonase activity and related lipoproteins in familial hypercholesterolemic patients. *Arterioscler. Thromb. Vasc. Biol.* (2000) 2: 2113-9

(10) Superko HR, Haskell WL and Krauss RM. Association

of lipoprotein subclass distribution with use of slective and non-selective beta-blocker medication in patients with coronary heart disease. *Atherosclerosis* (1993) 101: 1-8

(11) Dantoine TF, Debord J, Chames JP, Marquet P, Marle L, Lachatre G and Leroux-Robert C. Decrease of serum paraoxonase activity in chronic renal failure. *J. Am. Soc. Nephrol.* (1998) 9: 2082-88

(12) Mackness B, Durrington PN and Mackness MI. Human Serum Paraoxonase. *Gen. Pharmac.* (1998) 31: 329-36

(13) Blatter M, James RW, Messmer S, Barja F and Pometta D. Identification of a distinct human high-density lipoprotein subspecies defined by a lipoprotein-associated protein, K-45: identity of K-45 with paraoxonase. *Eur. J. Biochem.* (1993) 211: 871-879

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