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Research Article

Inhibition effects of commonly used some antibacterial and antiviral drugs on purified human serum paraoxonase-1 (hPON1)

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ABSTRACT

hPON1 is an enzyme from the group of A-esterases which is capable of hydrolyzing the active metabolite paraoxon of parathion, an organic phosphorus insecticide. It is an important liver enzyme that plays a protective role against the hydrolysis of HDL-induced organophosphate agents and nerve gases, oxidation of LDL, formation of lipid peroxides and bacterial endotoxins. The fact that oxidation of LDL constitutes the initial stage of the atherosclerosis process reveals the importance of the antioxidant properties of the enzyme. In this study, human serum PON1 was purified using three simple biochemical purification techniques. Furthermore, the in vitro effects of some antibacterial and antiviral drugs on human serum PON1 enzyme activity were examined. IC50 values were determined.

Keywords: Paraoxonase, enzyme inhibition, antibacterial drug, antiviral drug.

Sık kullanılan bazı antibakteriyel ve antiviral ilaçların saflaştırılmış insan serum paraoksonaz-1 (hPON1) üzerine inhibisyon etkileri

ÖZ

hPON1, bir organik fosfor insektisid olan parathion'un aktif metabolit paraoksonunu hidrolize edebilen A-esteraz grubundan bir enzimdir. HDL'nin neden olduğu organofosfat ajanlarının ve sinir gazlarının hidrolizine, LDL'nin oksidasyonuna, lipid peroksitlerin ve bakteriyel endotoksinlerin oluşumuna karşı koruyucu bir rol oynayan önemli bir karaciğer enzimidir. LDL'nin oksidasyonunun ateroskleroz sürecinin başlangıç aşamasını oluşturması, enzimin antioksidan özelliklerinin önemini ortaya koymaktadır. Bu çalışmada, insan serumu PON1, üç basit biyokimyasal saflaştırma tekniği kullanılarak saflaştırıldı. Ayrıca, bazı antibakteriyel ve antiviral ilaçların insan serumu PON1 enzim aktivitesi üzerindeki in vitro etkileri incelendi. IC50 değerleri belirlendi.

Anahtar Kelimeler: Paraoksonaz, enzim inhibisyonu, antibakteriyel ilaç, antiviral ilaç.

1. INTRODUCTION

The relationship of bacteria and viruses to humans is as old as human existence. They cause various infections in children, young and old. Clarithromycin (Figure 1a) is a broad spectrum antibacterial drug. It is most commonly used for the treatment of respiratory infections.[1] In addition to its antibacterial activity, it shows a wide range of pharmacological effects.[2] Cefepime hydrochloride (Figure 1b) is a bactericidal cephalosporin for a wide variety of organisms. Cefepime hydrochloride which is a 4th generation cephalosporin is used widely as an antibacterial agent. It is effective against many types of bacteria.[3] Acyclovir (Figure 1c) is the most widely used powerful antiviral drug. It is used to inhibit replication of many viruses.[4] Cefazolin sodium (Figure 1d) is a antibacterial drug classified as first generation cephalosporin. It is a semi-synthetic β-lactam for intravenous administration. It has a limited spectrum of destructive bacteria. It is used for prophylaxis in pregnancy interventions.[5] hPON1 is a Ca^{2+} - dependent. It is called arildalkylalkyl phosphatase. It has been previously thought to be lactone because of its physiological effects, it has been found that the enzyme has many substrates and many functions. As a result of studies on mice, hPON1 enzyme has been determined to

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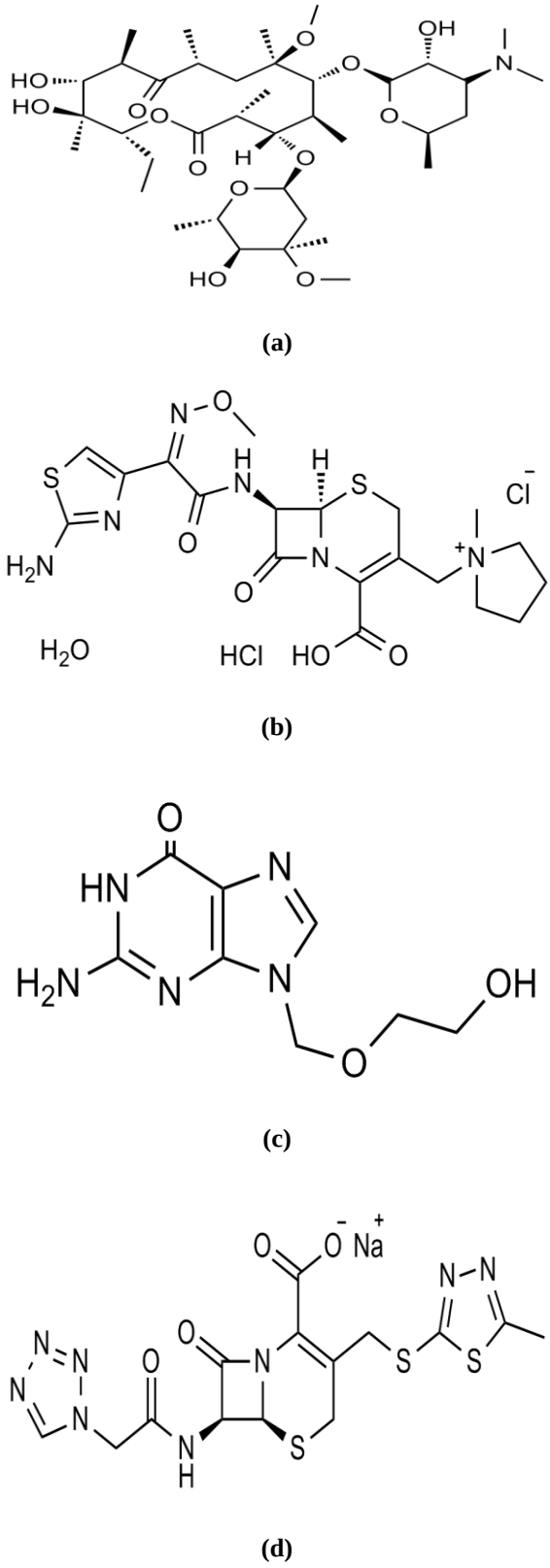

(a)

(b)

(c)

(d)

Figure 1. Chemical structures of the drugs: a) Clarithromycin, b) Cefepime hydrochloride, c) Acyclovir, d) Cefazolin sodium.

be an HDL dependent lactonase with antioxidant and antiatherogenic effects. It has been observed that the development of atherosclerosis is accelerated and oxidative stress is increased in mice lacking PON1 enzyme. It has been observed that hPON1 enzyme transferred mice has decreased both oxidative stress and the number of lesions.[6]

It has been found that it decreased lipid peroxide accumulation on LDL by an enzymatic mechanism when stored under HDL oxidizing conditions. hPON1 is one of the factors associated with this feature of HDL. Furthermore, hPON1 limits the accumulation of oxidized LDL and HDL particles. It inhibits the conversion of LDL to proatherogenic particles and reverses the biological effects of oxidized LDL particles. Thus, it has been shown to prevent the formation and progression of atherosclerosis lesions.[7]

Due to diseases affected by the change in hPON1 activity, the inhibition of antibacterial and antiviral drugs is important. If any drug causes a decrease in hPON1 enzyme activity, many vascular diseases may occur, including atherosclerosis. Indeed, due to the physiological role of hPON1, further studies on the inhibitory effects of drugs should be conducted. However, there are many studies about the inhibition effects of drugs on the hPON1.[7-9]

Antibacterial and antiviral drugs are used in the treatment of serious bacterial diseases. However, the patient is extremely important for adjusting drug doses. In this study, we purified hPON1 from human serum and examined the in vitro effects of antibacterial and antiviral drugs on enzyme activity.

2. MATERIALS AND METHODS

2.1. Materials

DEAE-Sephadex A50, Sepharose 4B, 1-naphthylamine, paraoxone, protein reagents and chemicals used for electrophoresis were obtained from Sigma Chemical Co. All other chemicals were obtained from Sigma-Aldrich or Merck. Clarithromycin, cefepime hydrochloride, acycloviron, and cefazolin sodium were obtained from local pharmaceutical manufacturing companies.

2.2. Paraoxonase activity assay

Human serum samples were supplied from the Research Hospital at Ataturk University. PON1 activity was determined at 250°C with paraoxon (diethyl p-nitrophenyl phosphate) (1mM) in 50 mM glycine/NaOH (pH 10.5) containing 1 mM $CaCl_2$. PON1 assay was based on the estimation of p-nitrophenol at 412 nm.

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The molar extinction coefficient of p-nitrophenol (ε = 18.29 $M^{-1}cm^{-1}$ at pH 10.5) was used to calculate PON1 activity. One enzyme unit was defined as the amount of enzyme that catalyses the hydrolysis of 1 µmol of substrate at 25°C. Assays were performed using a spectrophotometer (CHEBIOS UV-VIS).

2.3. Ammonium sulphate precipitation

Human serum precipitated with 60–80% ammonium sulphate was carried out in our previous studies. The precipitate was obtained after centrifugation at 15,000 × g for 20 minutes and redissolved in a 100 mM Na-phosphate buffer (pH 7.0).

2.4. DEAE-Sephadex A50 anion exchange chromatography

At first, the anion exchange column was equilibrated with a 100 mM Na-phosphate buffer (pH 7.0). Then, the enzyme solution, which had been dialyzed in the presence of 1 mM Na-phosphate buffer (pH 7.0) for two hours, was loaded onto the DEAE-Sephadex A50 anion exchange column (3 cm × 30 cm). Later, the chromatography column was washed with a 100 mM Na-phosphate buffer (pH 7.0), and then elution was carried out by an increasing linear gradient of 0-1.5 M NaCl. The elution fractions collected were checked for enzyme activity at 412 nm. Tubes which displayed the same enzyme activity were combined. All these procedures were performed at 4°C.

2.5. Sephadex G-200 gel filtration chromatography

In the first process, the sephadex G-200 column (60 cm x 2 cm) was equilibrated with 100 mM Na-phosphate buffer (pH 7.0). Fractions from the DEAE-Sephadex A50 anion exchange column were mixed with glycerol and loaded onto the gel filtration column with the same buffer. Finally, the enzyme solutions were separated from the sephadex G-200 column. Protein amount (at 280 nm) and enzyme activity (at 412 nm) were recorded for all tubes. Tubes showing enzyme activity were combined for other kinetic studies.

2.6. Protein Determination

Quantitative protein determination was performed by spectrophotometric purification steps at 595 nm according to Bradford method.

2.7. SDS polyacrylamide gel electrophoresis

SDS polyacrylamide gel electrophoresis was performed to check that the enzyme was purified according to the procedure of Laemmli. The resulting single band was photographed after electrophoresis.

2.8. In vitro studies for the drugs

We studied the inhibitory effects of four antibiotic drugs: Clarithromycin, cefepime hydrochloride, acycloviron, and cefazolin sodium. All compounds were tested three times for each concentration used. hPON1 activities were measured in the presence of different drug concentrations. In the absence of an inhibitor, the control activity was assumed to be 100%. For each drug, activity % - [I] plots were plotted and IC50 values were calculated from the equation of the curve.

3. RESULTS AND DISCUSSION

hPON1 is a calcium-dependent esterase that hydrolyzes esters such as organophosphate and lactone. hPON1 is a glycoprotein with a molecular weight of 43-45 kDa. It is mainly synthesized by the liver. hPON1 is one of the antioxidant defense mechanisms in the human body. There are many cleaning systems for reactive oxygen species, including paraoxones in the human body. hPON1 removes reactive oxygen species in living metabolism and protects LDL, HDL and macrophages against oxidative stress. Therefore, hPON1 prevents cardiovascular disease.[10-13]

There are many studies on hPON1 inhibition. For instance, Ekinci and Beydemir researched the effects of some drugs, lornoxicam (a), indomethacin (b), tenoxicam (c), diclofenac sodium (d), ketoprofen (e) and lincomycine (f), on hPON1 activity from human serum. They founded that lornoxicam inhibits the enzyme activity significantly, compared to the other drugs. The inhibition order of the drugs determined as a > b > c > d > e > f.[14] In another study, the impacts of some commonly used antibiotics as teicoplanin, rifamycin, tobramycin, ceftriaxone sodium, cefuroxime sodium, ceftazidime, ornidazole, and amikacin sulfate were investigated on human serum hPON1 activity. The major inhibitor was teicoplanin.[15]

For example, Türkeş and co-workers have investigated that some chemotherapeutic drugs, palonosetron hydrochloride, bevacizumab, and cyclophosphamide on hPON1 effects. Inhibition range of the drugs are in the order palonosetron hydrochloride > bevacizumab > cyclophosphamide. However, chemotherapeutic drugs are used to treat cancer patients. Therefore, this may be particularly critical for patients with atherosclerotic lesions.[9] For instance, Türkeş and co-workers have reported that some antibacterial drugs, moxifloxacin hydrochloride (1), levofloxacin hemihydrate (2), cefepime hydrochloride (3), cefotaxime sodium (4) and ceftizoxime sodium (5) on hPON1 effects. It is important that antibiotic drugs are potent inhibitors of human serum hPON1.

Moxifloxacin hydrochloride was found to significantly inhibit enzyme activity compared to other antibiotics.

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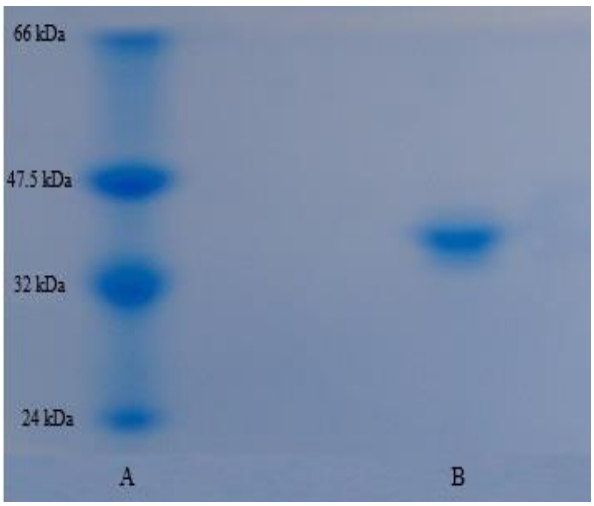

Figure 2. SDS-PAGE analysis of purified hPON1. Lane A is standard proteins (kDa): Bovine serum albumin (66 kDa), aldolase (47.5 kDa), triosephosphate isomerase (32 kDa), and soy bean trypsin inhibitor (24 kDa). Lane B contains a human serum sample.

Table 1. IC50 values

Inhibitors	IC_{50} (mM)
Clarithromycin	0.459
Cefepime hydrochloride	14.518
Acycloviron	56.169
Cefazolin sodium	150.602

The order of inhibition of the drugs was determined as 1 > 2 > 3 > 4 > 5.[8]

In this study, a specific activity of 4060 EUx mg^{-1} from hPON1 human serum was purified approximately 295-fold in 53.9% yield.

The purified human serum paraoxonase had a molecular weight of 43 kDa (Figure 2). Inhibition effect studies on paraoxonase activity of antibiotic drugs were performed. IC50 values for (a) clarithromycin, (b) cefepime hydrochloride, (c) acycloviron, and (d) cefazolin sodium were determined by means of activity % - [I] graphs as 0.459, 14.518, 56.169, and 150.602 mM, respectively (see Table 1 and Figure 3). As a result of inhibition studies, it was observed that antibiotic drugs inhibit hPON1 enzyme. Clarithromycin showed the most inhibition.

It is known that an adult person has a total of about 5 liters of blood. Accordingly, cefepime hydrochloride (b), acycloviron (c), and cefazolin sodium (d) blood concentrations were calculated as 0.175, 0.0198, and

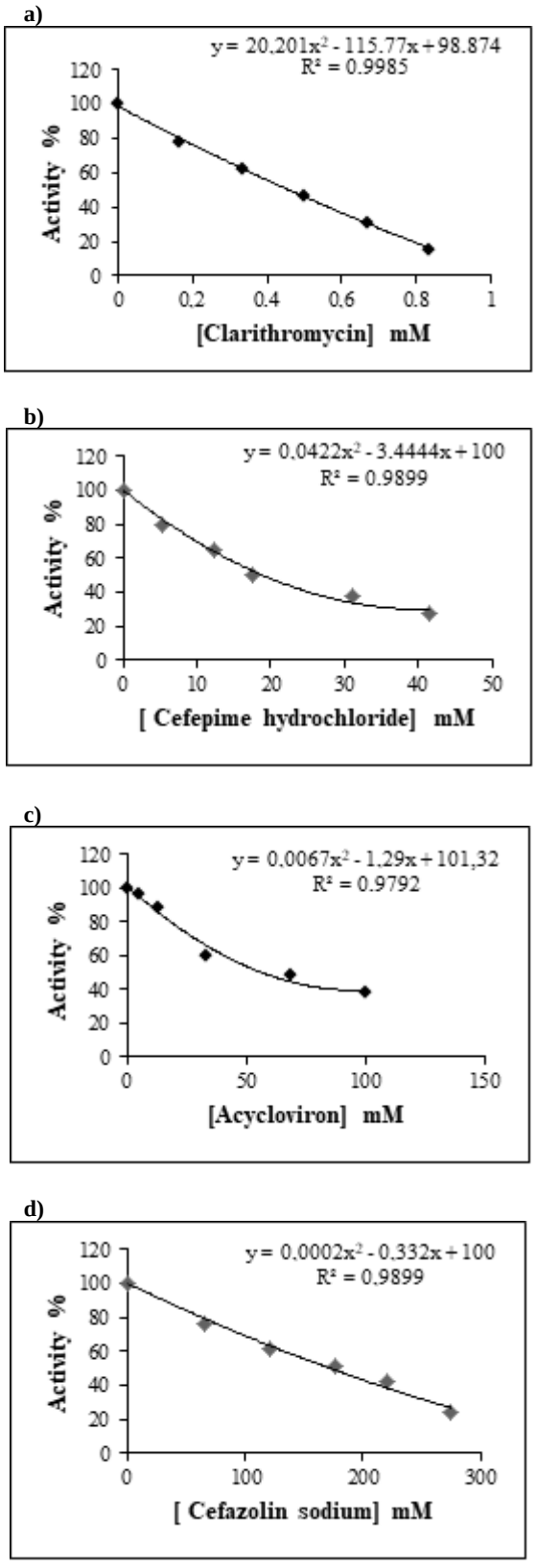

Figure 3. *In vitro* effect of antibacterial drugs: **a)** clarithromycin, **b)** Cefepime hydrochloride, **c)** Acycloviron, **d)** Cefazolin sodium at five different concentrations on hPON1 activity.

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0.440 mM, respectively. These values are lower than IC50 values. However, (a) the blood concentration of clarithromycin was determined to be 0.134 mM near the IC50. This research can be clarified by in vitro studies. Because these antibacterial drugs are widely used in the treatment of bacterial infections.

4. CONCLUSIONS

As a result, hPON1 was purified using three simple purification steps. The in vitro effects of antibacterial drugs on hPON1 were investigated. Blood concentrations and IC50 drug values were then compared. However, clarithromycin is the most inhibition drug. Prolonged use of this drug may cause a decrease in enzyme activity. Decrease in enzyme activity may contribute to atherosclerosis. Consequently, it may increase cardiovascular diseases.

Conflict of interest

Author declares that there is no a conflict of interest with any person, institute, company, etc.

REFERENCES

1. Bandettini di Poggio, M.; Anfosso, S.; Audenino, D.; Primavera, A. *J. Clin. Neurosci.* **2011**, 18, 313-318.

2. Giamarellos-Bourboulis, E.J.; Antonopoulou, A.; Raftogiannis, M.. Koutoukas P, Tsaganos, T., Tziortzioti, V., Panagou, C., Adamis, T., Giamarellou, H. *BMC Infect. Dis.* **2006**, 21, 31.

3. Cheathama, S. C.; Shea, K. M.; Healy, D. P.; Humphrey, M. L.; Fleming, M. R.; Wack, M. F.; Smith, D. W.; Sowinski, K. M.; Kays, M. B. *Int. J. Antimicrob. AG.* **2011**, 37 (1), 46-50.

4. Feng, M.Q.; Cai, Q.S.; Huang, H.; Zhou, P. *Eur. J. Pharm. Biopharm.* **2008**, 68, 688-693.

5. Singh, A. K.; Quraishi, M. A. *Corros. Sci.* **2010**, 52, 152-160.

6. Khersonsky, O.; Tawfik, D. S. *Biochemistry* **2005**, 44, 6371-6382.

7. Türkeş, C.; Söyüt, H.; Beydemir, Ş. *Pharmacol. Rep.* **2014**, 66, 74-78.

8. Türkeş, C.; Söyüt, H.; Beydemir, Ş. *J. Enzyme Inhib. Med. Ch.* **2015**, 30, 622–628.

9. Türkeş, C.; Söyüt, H.; Beydemir, Ş. *Environ. Toxicol. Phar.* **2016**, 42, 252-257.

10. Gaidukov, L.; Tawfik, D. S. *Biochemistry* **2005**, 44, 11843-11854.

11. Renault, F.; Chabriere, E.; Andrieu, J.P.; Dublet, B.; Massona, P.; Rochua, D. J. *Chromatogyr. B*, **2006**, 836, 15-21.

12. Aviram, M.; Rosenblat, M. *Free Radical Bio. Med.* **2004**, 37, 1304–1316.

13. Rozenberg, O.; Shih, S. D.; Aviram, M. *Atherosclerosis* **2005**, 181, 9–18.

14. Ekinci, D.; Beydemir, S. *J. Enzyme Inhib. Med. Ch.* **2009**, 24, 1034-1039.

15. Ekinci, D.; Beydemir, S. *Eur. J. Pharmacol.* **2009**, 617, 84-89.

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