

Investigation effects of some anthraquinones on human paraoxonase 1 (hPON1)

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Abstract

The *in vitro* effects of the some dihydroxyanthraquinone derivates, anthrorufin, xanthopurpurin, chrysazin and chrysaphanol which are commonly used as anticancer antibiotics, on the activity of purified serum paraoxonase 1 (PON1) (EC 3.1.8.1.) were investigated. Paraoxonase 1 was purified from human blood serum 355,12 fold with 55 % yield with use of ammonium sulphate precipitation and Sepharose-4B-L-tyrosine-1-naphthylamine HIC (hydrophobic interaction chromatography) coloumn in return. Sodium Dodecyl Sulphate -polyacrylamide gel electrophoresis (SDS-PAGE) analysis of purified enzyme showed a single band belong to human serum PON1, 43 kDa. The four anthraquinones inhibited PON activity strongly, with IC_{50} values for anthrorufin, xanthopurpurin, chrysazin and chrysaphanol of 73, 72.25, 75.19 and 105.53 μM respectively. K_i constants were determined 34.64, 39.51, 39.73 and 63.59 μM , respectively. All anthraquinones showed inhibition in competitive manner.

Keywords: Paraoxonase, inhibition, anthraquinones.

Bazı antrakinonların insan paraoksonaz 1 (hPON1) üzerine etkilerinin incelenmesi

Özet

Antikanser antibiyotikler olarak yaygın kullanılan bazı dihidroksiantrakinon türevlerinin (antrorufin, ksantopurpirin, krisazin ve krizofanol) saflaştırılmış serum paraoksonaz 1 (PON1) aktivitesi üzerine (EC 3.1.8.1) *in vitro* etkileri araştırıldı. Paraoksonaz 1, insan kan serumundan 355,12 kat, % 55 verimle, amonyum sülfat çöktürmesi ve Sepharose 4B-L-tirozin-1-naftilamin hidrofobik etkileşim kromatografisi kullanılarak saflaştırılmıştır. Saflaştırılmış enzimin Sodyum Dodesil Sülfat-

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poliakrilamid jel elektroforezi (SDS-PAGE), insan serum PON1'e ait 43 kDa'lık tek bant gösterdi. Dört antrakinon bileşiği, PON1 aktivitesini, sırasıyla antrorufin, ksantopurpirin, krisazin ve krizofanol için 73, 72.25, 75.19 ve 105.53 μ M değerleri ile güçlü bir şekilde inhibe etmiştir. Ki sabitleri sırasıyla 34.64, 39.51, 39.73 ve 63.59 μ M olarak bulunmuştur. Tüm antrakinonlar yarışmalı bir şekilde inhibisyon göstermiştir.

Anahtar kelimeler: Paraoksonaz, inhibisyon, antrokinonlar.

1. Introduction

There has been a growing interest to understand physiological functions of multiple functional paraoxonase 1 (PON1) in last decades. PON1 is one of HDL associated enzymes, both capable of inhibiting LDL and cellular oxidation [1]. In addition recent studies show PON1 prevents infection cause of quorum sensing bacteria via its lactonase activity [2]. So that paraoxonase 1 play role in several type diseases such as; atherosclerosis, diabetes, inflammation, immune system disorders, ageing and cancer [3].

Also human serum paraoxonase-1 (PON1) capable of catalyzing a wide range of substrates; aryl esters, cyclic carbonate esters, aromatic esters, lactones, number of pharmaceutical agents, thiolactones, organophosphates, highly toxic nerve gases (sarin and soman) [4].

X-ray crystallography show that the structure of PON1 has three carbohydrate chains where in two calcium ions, one essential for catalytic, the other one for structural. Paraoxonase1 is a glycoprotein that consists of 354 amino acids with 43 kDa molecular weight. Human serum paraoxonase-1 (hPON1), produced mainly in the liver then secreted into blood serum connected to high density lipoprotein (HDL), which also contains ApoA1 [5].

Paraoxonase (PON) gene has three isoenzymes, PON1, PON2 and PON3, located next to each other on chromosome 7q21.3-22 in humans (6 in mice), which are all widely expressed in mammalian tissues [6]. The PON1 promoter region has two binding sites both specificity protein 1 (Sp-1), which approximately up regulate PON1 in the presence of statins and sterol regulatory binding protein 2 (SREBP2). PON1 has many regulatory factors; listed as diet, smoking, inflammation and diabetes [7].

However there has little studies about paraoxonase's physiological properties still in the literature. For this reason there are needed drug interaction experiments on PON1 much more. In present study it was investigated in vitro the effects of four different dihydroxy anthraquinone derivates (Figure 1) on purified human serum paraoxonase 1 activity.

Anthraquinones (AQs) are aromatic compounds which both synthetically occurring from quinones and naturally occurring from fungi, lichen, root. AQ or derivatives are using as dyestuffs in foods, drugs, and cosmetics [8,9]. As well as the importance of the AQs cause of widely applications in commercial, they also demonstrate many biological functions including bone cancer therapy, antienflamatuar, antimicrobial, antigenotoxic, phytoestrogen, diuretic and vasorelaxing through the literature. However, there is need

to be more information about biological properties [10,11]. From this point of view, in this study focused on biological effects of these anthraquinones (anthrorufin, xanthopurpurin, chrysazin and chrysaphanol) via interactions with PON1.

2. Experimental

1.1. Materials

The compounds used in this research, consist of Sepharose 4B, CNBr, 1-naphthylamine, paraoxon-ethyl, chemicals for electrophoresis and anthraquinones (anthrorufin, xanthopurpurin, chrysazin and chrysaphanol) were purchased from Sigma Chemical Co or Merck. For the inhibition studies, it was used a BIOTEK UV-VIS spectrophotometer.

1.2. Purification of human serum paraoxonase 1 from chromatographic method

50 ml fresh human blood was put into centrifuge tube. The blood sample was centrifuged at 5000 rpm for 20 min and serum was isolated. Firstly, serum was precipitated with 60-80 % ammonium sulphate and the fractionation was collected by centrifugation at 15000 rpm for 45 time, and dissolved in 1 M ammonium sulphate (pH 8.0). Secondly, it was used the hydrophobic gel Sepharose 4B-L-tyrosine-1-naphthylamine which synthesized in our laboratory before [12], for purification of paraoxonase1. The column was equilibrated with buffer mix consist of 0.1 M Na₂HPO₄ (pH 8.00) and 1 M (NH₄)₂SO₄ (pH 8.00). The paraoxonase was eluted and purified PON1 enzyme was kepted in +4 °C.

1.3. Paraoxonase enzyme activity assay

The enzyme activity was studied at with paraoxon substrate (2 mM diethyl p-nitrophenyl phosphate) and 0.1 M Na₂HPO₄ buffer (pH 8.00). The paraoxonase enzyme assay was obtained from paraoxon's hydrolysis to p-nitrophenol at 412 nm at 37 °C [13]. The assays were determined via BIOTEK UV-VIS spectrophotometer.

1.4. Ammonium sulphate precipitation

Human serum paraoxonase was precipitated with 60–80 % (NH₄)₂SO₄, as in previous study [14]. The precipitate was obtained after centrifugation at 15000 rpm for 50 min and dissolved in 1 M (NH₄)₂SO₄ buffer (pH 8.00).

1.5. Protein determination

During the purification steps, quantitative protein assay was determined according to the Bradford method at 595 nm by spectrophotometer [15].

1.6. SDS-PAGE gel electrophoresis

SDS-PAGE gel electrophoresis was applied according to Laemmli's procedure [16] to purified hPON1 from HIC column including Sepharose 4B-L-Tirozin-1-naptylamine. The single protein band belong to paraoxonase 1 was photographed after electrophoresis (Figure 2).

1.7. in vitro drug studies and calculation of kinetic values

It was performed the inhibitory efficacy of four different anthraquinone derivates including anthrorufin, xanthopurpurin, chrysazin and chrysaphanol on paraoxonase 1. At each concentration used, all measurements were repeated three times. The PON1

activities were measured in the presence of different compounds concentrations. The control activity was assumed to be 100 % in the absence of an inhibitor. For each anthraquinone compounds, the percent activity graph as a function of the drug concentration was drawn in excel (Figure 3). To determine the values of K_i , two different inhibitor concentrations were examined for each dye. All these experiments, paraoxon was used as substrate at different concentrations. The resulting Lineweaver–Burk curves were used to determine the value of K_i and the inhibitor type (Figure 4) [17].

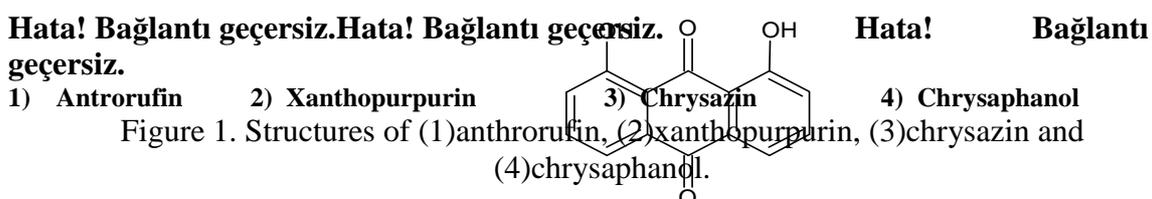
3. Results and discussions

Human PON1 was purified by 1-naphthylamine-L-tyrosine-Sepharose 4B gel affinity column [12]. Specific activity was calculated for the serum and ammonium precipitation purified enzyme solution as 65.71 EU/mg protein, a yield of 45 % and a purification coefficient of 355.12-fold. Purification steps were controlled by SDS-PAGE (Figure 2).

The number of studies with paraoxonase inhibition has increased in the last decade [18-20]. In this study four anthraquinones compounds showed potent inhibitory effects on paraoxonase activity. The obtained IC_{50} values for anthrorufin, xanthopurpurin, chrysazin and chrysaphanol were 72.25, 73, 75.19, and 105.53 μ M, respectively (Table 1). K_i values were calculated from Lineweaver-Burk graphs. K_i constants for the drugs were 34.64, 39.51, 39.73 and 63.59 μ M respectively (Table 1). Inhibition types were found as competitive for the all dyes (Table 1).

Anthraquinones are the most general group, naturally derivate from quinones. Synthetically and natural AQs are using as dyestuffs in food, drugs, and cosmetic areas [8-11]. However, there is really very little information about biological functions of these compounds. Anthrarufin is a natural anthraquinone, occurring from some kind of fungi and bacteria. Xanthopurpurin obtained from tea, herbs and spices. Chrysazin found in dried leaves and stems of *Xyris semifuscata*, a specie of plant in Madagascar [21]. Chrysophanol like anthrarufin extracted from fungi, also called chrysophanic acid [22- 24].

Anthraquinones have ability of binding calcium makes AQs useful in biochemical studies involving bone growth and osteoporosis, gene expression and tissue engineering [21]. Human serum PON1 is a calcium-dependent enzyme that hydrolyzes esters such as organophosphate and lactone. Anthraquinones may be able to inhibit the enzyme by complexing with the calcium required for enzyme activity.



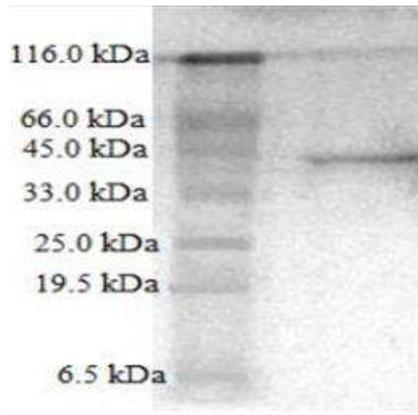


Figure 2. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) analysis of purified human serum paraoxonase 1 (hPON1).

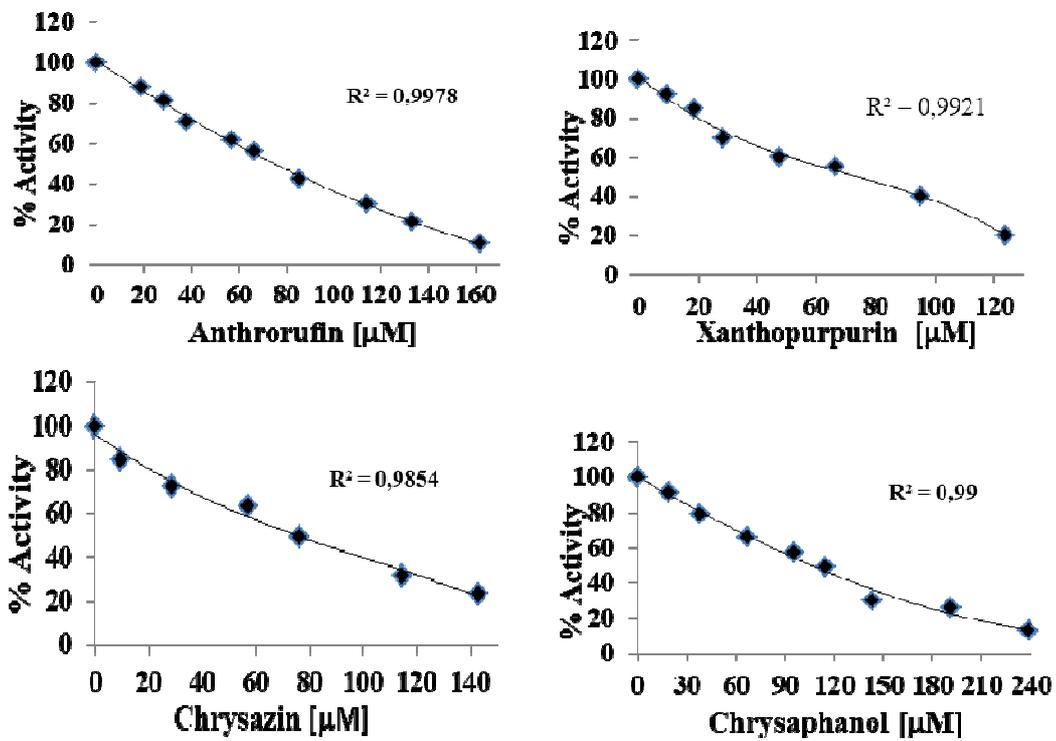


Figure 3. Investigation of IC_{50} values of each AQ compounds obtained by drawn % Activity against inhibitor concentration graphs in Excel.

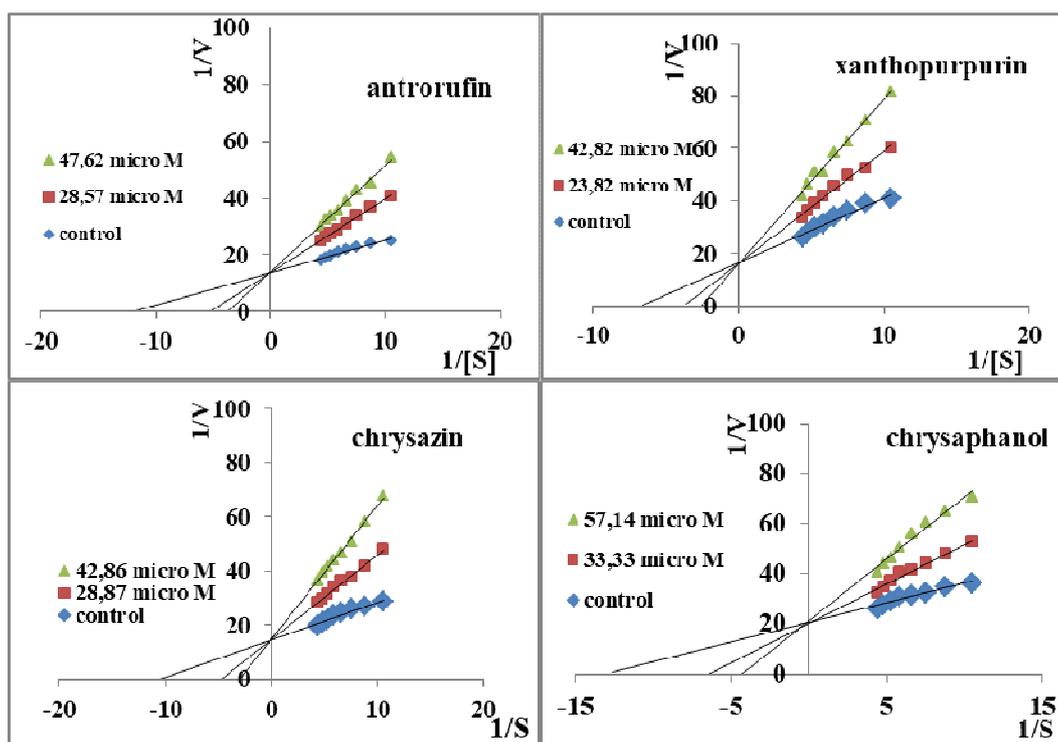


Figure 4. Investigation of inhibition types and K_i values of the AQs by using Lineweaver–Burk curves.

Table 1 . IC_{50} , R^2 , K_i values and inhibition types for four AQ derivatives.

| Anthraquinones | IC_{50} (μ M) | R^2 | K_i (μ M) | Inhibition type |
|----------------|----------------------|-------|------------------|-----------------|
| Anthrorufin | 73 | 0.998 | 34.64 | Competitive |
| Xanthopurpurin | 72.25 | 0.992 | 39.51 | Competitive |
| Chrysazin | 75.19 | 0.985 | 39.73 | Competitive |
| Chrysaphanol | 105.53 | 0.990 | 63.59 | Competitive |

4. Conclusions

In conclusion, it was purified human serum PON1 enzyme using two simple purification steps and investigated the in vitro effects of some AQs, namely anthrorufin, xanthopurpurin, chrysazin and chrysaphanol on the enzyme activity.

These naturally occurring AQs have been used in therapeutic applications such as, anticancer, anti-inflammatory, antimicrobial, diuretic, and phytoestrogen activities like many diseases. PON1 is critical in cardiovascular diseases and detoxification metabolism. Due to this reason, it is important to examine the effects of these compounds interaction with PON1 activity. All anthraquinones compounds inhibited the enzyme activity potently in competitive manner. Studies on the effects of drugs on the paraoxonase 1 enzyme should be investigate more also in clinical and genetic.

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