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Research Article
Inhibitory Effect of Methotrexate (MTX) Used in Human Cancer Treatment on Paraoxonase-1 (PON1)

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Abstract

Background and Objective: Paraoxonase-1 (PON1) is a lactonase that plays a role in the destruction of carcinogenic free radicals and in reducing oxidative stress. In this article, it was aimed to investigate the inhibitory effect of methotrexate (MTX) on PON1. **Materials and Methods:** In this study, the inhibition between methotrexate used in cancer chemotherapy and PON1 was studied. **Results:** Methotrexate showed very strong inhibitory properties for human serum PON1, with an IC_{50} value of 38.50 μM and a mean K_i value of 42.36 μM. Hence methotrexate showed competitive inhibition. **Conclusion:** The widespread use of methotrexate, a dihydrofolate reductase (DHFR) inhibitor, in the treatment of human cancers can be very harmful.

Key words: PON1, methotrexate, inhibition, anticancer drug, oxidative stress, LDL, atherosclerosis

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Methotrexate (MTX) is a structural analogue of folic acid and a dihydrofolate reductase (DHFR) inhibitor. It is a widely used anti-cancer agent for human leukemia, severe psoriasis and some solid tumors. The MTX can arrest intracellular folate metabolism and finally block pyrimidine and purine synthesis, leading to disruption of tumor growth and induction of cell death through apoptosis[1]. The antioxidant system defense developed against reactive oxygen species (ROS) and ROS in the organism is balanced. Oxidative stress (OS) is defined as an imbalance between free radical production and the antioxidant defense system[2]. One of the most important antioxidant defense systems against lipid oxidation is the paraoxonase-1 (PON1) enzyme. The PON1 enzyme prevents lipid peroxidation caused by oxidative stress. It also protects both low-density lipoprotein (LDL) and high-density lipoprotein (HDL) against the harmful effects of oxidative stress. Not only lipoproteins, but also lipids in the cell structure undergo lipid peroxidation by oxidative stress. The PON1 neutralizes the atherogenic effects of lipid peroxides and offers a protective effect on cellular membranes[3].

Paraoxonase-1 (PON1) was first discovered in mammals as an enzyme capable of hydrolyzing organophosphate pesticides. Later, this enzyme was classified as esterase. However, now PON1 is defined as an aryl dialkyl phosphatase (EC 3.1.8.1)[4]. However, this enzyme is commonly referred to as "paraoxonase" because it is commonly used to hydrolyze the harmful pesticide paraoxon. The PON1 hydrolyzes nerve gases such as lactones, thiolactones, arylesters, cyclic carbonates, highly toxic organophosphates, soman and sarin. However, the physiological substrate of PON1 is unknown[5]. The PON1 is synthesized in the liver and then secreted into the blood serum in conjunction with high-density lipoprotein-HDL. The PON1 is seen not only in the liver, but also in many mammalian tissues, including human tissues[6].

It is known that almost all drugs exert their effects on the organism by influencing enzyme activities. Such enzymes are called drug-targeted enzymes. Many proteins act as drug targets. This is an important clue for drug design and development studies. On the other hand, not only drugs, but also pesticides, various chemicals and environmental pollutants may have the ability to inhibit enzyme activity by binding to the active site. These compounds exert their biological effects through interaction with typical enzymes. Inhibition or induction of specific enzymes can cause toxicities and metabolic interactions[7-9]. In this respect, it is clear that people cannot be exposed to many chemicals, especially drugs. Therefore, it is vital to regulate the optimal doses of drugs used for both patients and diseases. In recent years, there has been an increasing interest in understanding the physiological functions and kinetic properties of PON1. Thus, it plays an important role in various types of diseases such as diabetes medulla, inflammation and neuroprogressive disorders, which are associated with atherosclerosis in metabolism[10-12]. Apart from the vital functions of PON1, little is known about PON1-drug interaction.

In the light of the above information, current study aimed to investigate the inhibition effect of methotrexate, which is widely used as an anticancer drug, on human PON1 in this work.

MATERIALS AND METHODS

Study area: The study was carried out at Chemistry Department, Research Lab, Turkey from August, 2021 to October, 2021.

Chemicals: All chemicals were obtained from Sigma-Aldrich Chemie GmbH (Taufkirchen, Germany). Methotrexate (MTX) was obtained from Bursa Uludağ University Faculty of Medicine Oncology Department.

Paraoxonase activity measurement: Human serum samples were obtained from Erzincan Mengücekgazi Research Hospital. PON1 activity for paraoxon (diethyl p-nitrophenyl phosphate) was determined at pH 8.0 in 100 mM tris buffer at 37°C. Paraoxonase enzyme activity analysis was based on the prediction of p-nitrophenol at 412 nm. The PON1 activity was calculated using the molar extinction coefficient of p-nitrophenol (ϵ = 17100 M^{-1} cm^{-1}). Assays were analyzed with a Biotek automated recording spectrophotometer.

Determination of IC_{50} and K_i constants for anticancer drug: The inhibitory effect of methotrexate (MTX), which is widely used in cancer chemotherapy, was investigated. This anticancer drug was tested three times for each concentration. Paraoxonase activities of the enzyme were analyzed at different drug concentrations. Graphs were plotted showing percent activity for methotrexate as a function of drug concentration. Control activity in the absence of inhibitor was accepted as 100%. The 50% inhibition (IC_{50} value) of methotrexate was obtained from the graphs using different inhibitor concentration values. To calculate K_i values, three different inhibitory concentrations of methotrexate were tested Lineweaver-Burk curves were used to determine the values of K_i and the type of inhibition.

Statistical analysis: The mathematical relationship between the PON1 activity and inhibitor concentration was determined using conventional polynomial regression software (Microsoft Office 2010, Excel, Redmond, WA).

RESULTS

In this study, the *in vitro* inhibition effect of methotrexate on paraoxonase enzyme activity was studied. For methotrexate, which inhibits PON1, IC_{50} value (inhibitor concentration that halves the enzyme's activity) was determined by plotting percent activity-[Methotrexate] graphs using five different inhibitor concentrations at constant paraoxon concentration. The IC_{50} value was calculated as 38.50 μM from the curve equation in the graph (Fig. 1).

Then, 1/V-1/[Paraoxon] values were found by using the activity values obtained at three different inhibitor ([I1]:10, [I2]:30 and [I3]:50 μM) concentrations and five different paraoxon (1.33, 1.66, 2.22, 3.33 and 6.66 μM^{-1}) concentrations for methotrexate. The K_i values were calculated by drawing Lineweaver-Burk graphs. The average K_i value was found to be 42.36 μM (Fig. 2). The type of inhibition is competitive.

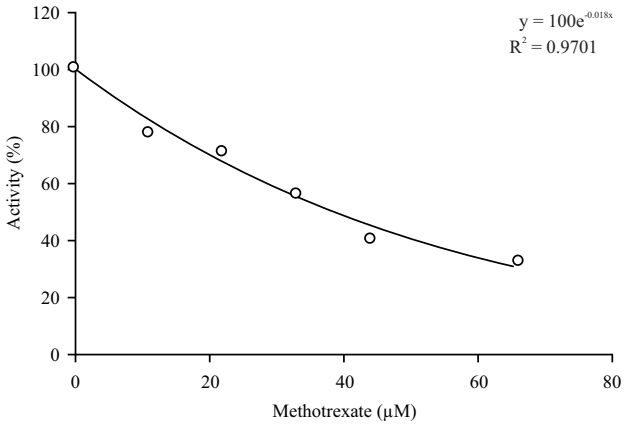

Fig. 1: Activity (%)-[concentration] graph used to determine the IC_{50} value

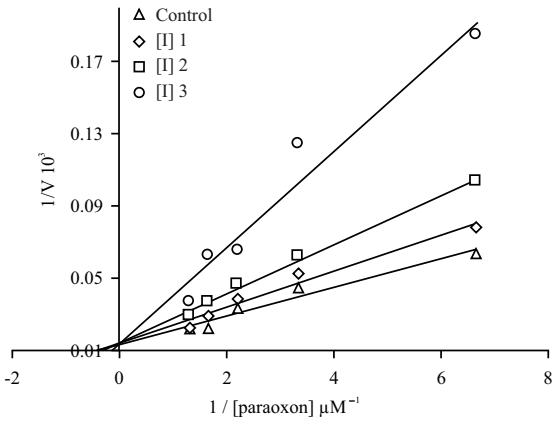

Fig. 2: Lineweaver-Burk graph used to determine K_i constant

DISCUSSION

In this study, methotrexate was tested on the paraoxonase activity of the PON1 enzyme and methotrexate inhibited PON1. The IC_{50} and K_i value for methotrexate were 38.50 μM and 42.36 μM, respectively. It interacted with active site residues of PON1 and exhibited competitive inhibition. Methotrexate inhibited the PON1 enzyme at a very low concentration, as it was a very potent inhibitor.

Most cancer drugs in the clinic act as enzyme inhibitors. Therefore, drug studies have focused on the characterization of drugs that inhibit enzymes[13]. *In vivo* or *in vitro* measurement of enzymatic activity has contributed greatly to drug discovery and development[14]. Although some research has been done on PON1, which is considered to be an important enzyme in the development of coronary artery diseases, there is little research on the effects of drugs on PON1 activity[15]. Researchers in different studies, the effects of calcium channel blockers, anticancer agents, antibacterial drugs and nucleoside analogues were investigated and the inhibition mechanisms of these drugs were determined. For example, Türkeş *et al.*[16] studied the inhibitory effect of palonosetron hydrochloride, bevacizumab and cyclophosphamide on paraoxonase activity. They found K_i constants of 0.033, 0.054 mM and 3.419 mM, respectively. When they compared the inhibition rates of the drugs, palonosetron hydrochloride had the maximum rate of inhibition[16]. In the study of the inhibition effects of calcium channel blockers (nifedipine, nitrendipine, isradipine and amlodipine besylate) on paraoxonase-1 (PON1), the drugs inhibited the enzyme activity at low concentrations. Nifedipine, nitrendipine, isradipine and amlodipine besylate showed inhibitory effects at low concentrations with IC_{50} values of 0.121, 0.130, 0.255 and 0.304 mM, respectively. The order of inhibitors (from strongest to weakest) was expressed as: Nifedipine>nitrendipine>isradipine>amlodipine besylate[17]. Alım and Beydemir investigated the effects of anticancer agents on PON1 enzyme activity. These drugs showed an inhibitory effect on the PON1 enzyme. The IC_{50} values were in the range of 0.011-23.3 mM[18]. In addition, Ekinci and Beydemir investigated the effects of various analgesic drugs on human PON1 activity. Lornoxicam, indomethacin, tenoxicam, diclofenac sodium, ketoprofen and lincomycin IC_{50} values were found as 0.13, 0.19, 0.34, 1.63, 6.23 and 9.63 mM,

respectively. These drugs were effective inhibitors of the PON1 enzyme. The authors also stated that the inhibition mechanisms of indomethacin and tenoxicam are competitive, ketoprofen is noncompetitive and lornoxicam, diclofenac sodium and lincomycin are uncompetitive[19]. In another study, the effect of antiepileptic drugs on PON1 was examined. Gabapentin, valproic acid, primidone, phenytoin and levetiracetam were found to decrease PON1 activity. It was determined that the inhibition types of all drugs were noncompetitive. According to the experimental results, gabapentin (IC_{50}: 0.35 mM and K_i: 0.261mM) was a potent inhibitor of the PON1 enzyme[20]. In an antibacterial study, Türkeş *et al.*[21] examined the inhibitory effect of moxifloxacin hydrochloride, levofloxacin hemihydrate, cefepime hydrochloride, cefotaxime sodium and ceftizoxime sodium on paraoxonase activity. They found K_i constants as 2.641, 5.525, 35.092, 252.762 and 499.244 mM, respectively. The mechanism of inhibition of moxifloxacin hydrochloride was competitive. Other drugs were non-competitive inhibitors[21]. In Soyut[22] study, busulfan, a chemotherapeutic drug, strongly inhibited PON1. As a result of this research, the IC_{50} and K_i values for busulfan were 77 µM and 42.83 µM, respectively. Busulfan showed competitive inhibition. All these drugs inhibited the PON1 enzyme.

According to the results of all the studies mentioned above, although the drugs used in the experiments were at therapeutic doses, they strongly inhibited PON1 and exhibited various types of inhibition. Compared with the other drugs mentioned above, methotrexate inhibited the enzyme more strongly than them. However, enzyme and drug interaction should be studied clinically. The inhibition relationship between methotrexate-PON1 can be clarified by *in vivo* studies.

CONCLUSION

As a result, it was determined that adjusting the dosage of this drug is a vital condition for each patient. Because if the individual has a low HDL level, the amount of PON1 decreases. This can cause various disorders such as coronary artery diseases and atherosclerotic lesions. The best way to avoid the side effects of drugs is not to use the drug unless there are serious symptoms or to use it at the recommended dose. As can be seen from the result, *in vitro* assay of methotrexate on PON1 is very important for drug concentration adjustment study and new drug discovery.

SIGNIFICANCE STATEMENT

This study discovered the therapeutic dose of methotrexate on PON1, which many researchers failed to discover. Thus, a new theory may have been reached on how methotrexate might motivate drug candidates to discover.

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