International Journal of Pharmacology

ISSN 1811-7775

science alert

ansinet
Asian Network for Scientific Information

OPEN ACCESS International Journal of Pharmacology

ISSN 1811-7775
DOI: 10.3923/ijp.2023.34.39

Research Article
Inhibition Effects of Oncological Drugs for Treatment of Cancer, on Paraoxonase-1 Enzyme (PON1) Activity and Cardiovascular Toxicity

Hakan Söyüt

Department of Primary Education, Faculty of Education, Bursa Uludağ University, Bursa, Turkey

Abstract

Background and Objective: Paraoxonase-1 Enzyme (PON1) is a high-density lipoprotein-associated cardioprotective enzyme that prevents the formation of oxidized low-density lipoprotein. This study focused on the investigation of the *in vitro* inhibition effects of trastuzumab and nivolumab, which are used in the treatment of cancer, on paraoxonase-1 enzyme activity. **Materials and Methods:** In this study, blood samples belonging to individuals aged 7-12 years were first taken and kept for the completion of coagulation. The serum was carefully separated by centrifugation. It was then used to investigate the inhibition effects of trastuzumab and nivolumab on the separated serum paraoxonase 1. In addition, the type of inhibition between enzymes and drugs was found by using Lineweaver-Burk curves. **Results:** Trastuzumab and nivolumab inhibited the human serum PON1 *in vitro* and their IC_{50} and K_i values were 0.0071 and 0.0062 μM, 0.0043 and 0.0053, respectively. Trastuzumab showed competitive inhibition. Nivolumab showed non-competitive inhibition. **Conclusion:** This study showed that trastuzumab and nivolumab inhibit PON1 enzymatic activity. These findings suggested that a decrease in the level of PON1 enzymatic activity may contribute to the increased risk of cardiovascular toxicity.

Key words: PON1, trastuzumab, nivolumab, inhibition, cardiovascular toxicity, melanoma, PON2

Citation: Söyüt, H., 2023. Inhibition effects of oncological drugs for treatment of cancer, on Paraoxonase-1 Enzyme (PON1) activity and cardiovascular toxicity. Int. J. Pharmacol., 19: 34-39.

Corresponding Author: Hakan Söyüt, Department of Primary Education, Faculty of Education, Bursa Uludağ University, Bursa, Turkey
Tel: +902242942204/+905537411369

Competing Interest: The author has declared that no competing interest exists.

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

INTRODUCTION

Cancer is a disease that kills millions of people every year around the world. Cancer treatment is one of the most challenging processes in medicine[1]. To date, a large number of drugs have been developed to treat cancer. Every year, scientists try to develop newer drugs with fewer side effects and higher efficacy[2]. However, chemotherapy drugs have a wide variety of side effects in the treatment of cancer. One of the most important side effects of cancer chemotherapy is cardiovascular toxicity[3-5]. Advances in cancer knowledge and treatment have led to a new field called cardiovascular oncology[6,7]. Trastuzumab (Herceptin, Genentech Inc., South San Francisco, CA, USA) is a monoclonal antibody that targets the human epidermal growth factor receptor 2 (HER2)[8]. Trastuzumab is considered a cornerstone in breast cancer treatment. It has been used for over 10 years to treat breast cancer[9,10]. Breast cancer is the second most common cancer worldwide and the most common cancer among women. Breast cancer patients overexpress HER2[11]. This type of cancer has been associated with accelerated tumour growth, early metastasis and therefore a poor prognosis. Trastuzumab significantly improved the survival of patients with breast cancer[12,13]. Nivolumab (ONO-4538, BMS-936558) is a human monoclonal immune checkpoint inhibitor (ICI) antibody that targets programmed cell death-1(PD 1). It binds specifically to (PD 1) in tumour tissue and inhibits the interaction between PD-1 and programmed cell death ligand 1 (PD-L1)[14]. Oncological studies with nivolumab have shown significant activity in metastatic melanoma, despite its cardiovascular toxic effects. Nivolumab has also proven efficacy in the treatment of many other types of cancer, such as bladder and breast cancer[15]. However, a very important issue regarding these oncological drugs is their cardiovascular toxicity side effects. Understanding the cardiovascular effects of treatment in breast cancer is becoming increasingly important. Because cardiovascular toxicity is the most common cause of death in these breast cancer patients, especially in elderly women[16-19].

Paraoxonase 1 (PON1) is a member of the gene family, which is encoded in three different places, including PON2 and PON3[20]. The location of the PON1 gene is between q22.1 and q21.3 on the long arm of human chromosome 7. According to the structural study using X-ray crystallography, PON1 has a six-bladed propeller structure and its central tunnel can accommodate two calcium ions[21]. PON1 (EC 3.1.8.1) is a blood-borne lactonase with a molecular weight of 43-45 kDa. It is synthesized in the liver and transported on High-Density Lipoprotein (HDL) particles after being secreted into the blood. Paraoxonase-1 (PON1) is a High-Density Lipoprotein (HDL)-related antioxidant enzyme that helps prevent the formation of oxidized Low-Density Lipoprotein (LDL) and reduces the development of atherosclerotic plaque. Atherosclerotic plaque formation may be directly proportional to cardiovascular toxicity[22]. The association of PON1 with cardiovascular toxicity was investigated in cancer patients treated with oncological drugs. PON1 activity was found to be lower in these patients. Numerous studies speculate that the reduction in PON1 enzymatic activity in patients with cancer is associated with cardiovascular toxicity induced by oncological drugs. Therefore, these results may cause cardiovascular toxicity by inhibiting the PON1 of these drugs used in cancer treatment. As a result, decreased PON1 activity may increase cardiovascular toxicity and plaque formation[23,24].

Drugs can show many toxicological effects on the organism by reducing enzyme activities. Therefore, the toxicity of drugs used for patients is of vital importance. In recent years, the effects of various drugs on paraoxonase activity have been investigated. Calcium channel blockers, anticancer agents and antiepileptic drugs play a role in PON1 inactivation[25-27]. There is increasing interest in documenting the link between PON1 and cardiovascular toxicity. Drugs that reduce PON1 activity may contribute to increased cardiovascular toxicity.

There were not many studies on the interactions between oncological drugs and PON1 activity. Hence, this study was conducted to investigate the *in vitro* inhibitory effects of some oncological drugs (trastuzumab and nivolumab), which were widely used in oncology, on this enzyme.

MATERIALS AND METHODS

Study area: The study was carried out in Department of Biochemistry, Research Lab, Turkey from March to September, 2022.

Chemicals: Paraoxon was obtained from Sigma-Aldrich (Germany). All other chemicals used were of analytical grade and purchased from either Sigma-Aldrich or Merck. Trastuzumab and nivolumab were obtained from the Department of Oncology, Faculty of Medicine, Bursa Uludağ University, Turkey.

Paraoxonase activity measurement: The PON1 activity was measured spectrophotometrically. Paraoxonase enzyme activity analysis was based on the prediction of p-nitrophenol at 412 nm. Paraoxon (diethyl p-nitrophenyl phosphate) was used as the substrate. PON1 activity was determined at 25 °C in 100 mM tris buffer pH 10.5. The molar extinction coefficient

of p nitrophenol is 18.290 M^{-1} cm^{-1}. P-nitrophenol was monitored for 2 min at 25°C. The reaction was carried out in an automatic registration spectrophotometer (Biotek, Winooski, VT).

Determination of IC_{50} and K_i constants for anticancer drug: For trastuzumab and paclitaxel inhibition studies, different concentrations of trastuzumab and nivolumab were added to the reaction medium. Oncological drugs and PON1 activities were tested by following paraoxon hydration. The PON1 enzyme activity was accepted as 100% activity without oncological drugs. Inhibitor concentrations (IC_{50} values) resulting in 50% inhibition for trastuzumab and nivolumab were calculated from the graphs. In addition, K_i values of oncological drugs were measured at pH 10.5 and 25°C using five different paraoxon concentrations. Five different substrate concentrations were added to the reaction medium in the presence and absence of three different inhibitor concentrations of oncological drugs and K_i values and inhibition types were determined using Lineweaver-Burk curves.

Statistical analysis: Conventional polynomial regression software (Microsoft Office 2010, Excel, Redmond, WA) was used for the mathematical relationship between PON1 activity and oncological drug concentrations.

RESULTS

In the current study, the *in vitro* inhibitory effects of trastuzumab and nivolumab on PON1 were determined. The IC_{50} values were obtained from a plot of activity (%) versus drug concentrations. The IC_{50} values were 0.0071 and 0.0043 μM, respectively (Fig. 1-2). Different concentrations of paraoxon were used to determine the K_i constants in the reaction medium in which trastuzumab and nivolumab were used as inhibitors. Lineweaver-Burk plots were drawn to determine the K_i values and inhibition types for oncological drugs. K_i values were found as 0.0062 and 0.0053 μM, respectively (Fig. 3-4). Trastuzumab showed a competitive inhibitory effect, while nivolumab showed a noncompetitive inhibitory effect.

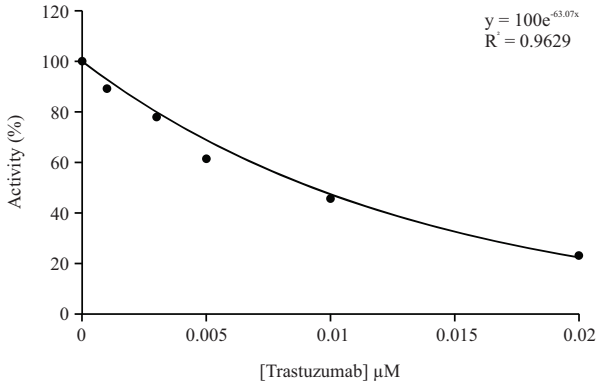

Fig. 1: Activity (%) (trastuzumab) graph used to determine the IC_{50} value

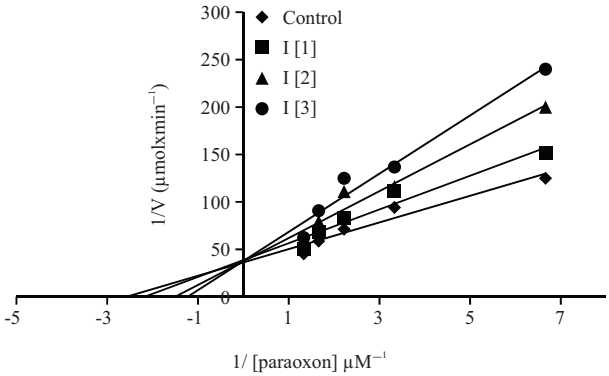

Fig. 2: Lineweaver-Burk graph used to determine K_i constant

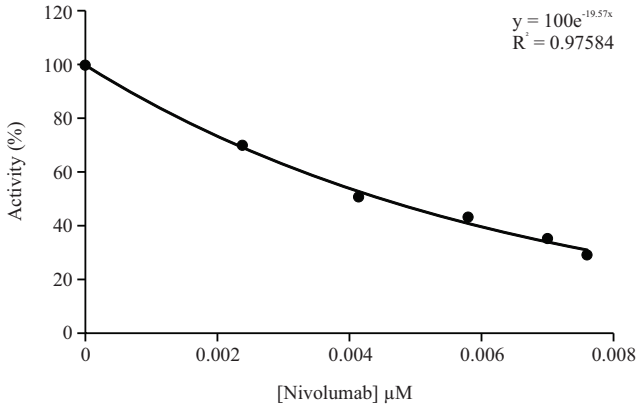

Fig. 3: Activity (%) (nivolumab) graph used to determine the IC_{50} value

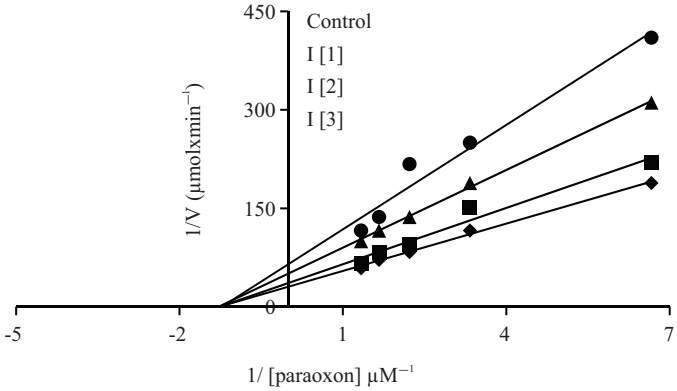

Fig. 4: Lineweaver-Burk graph used to determine K_i constant

DISCUSSION

This research aimed to define the relationship between trastuzumab and PON1 used as a biomarker. The main findings of this study were as follows: 1) PON1 enzyme activity was decreased with trastuzumab, 2) A decrease in PON1 activity may be associated with a higher risk of cardiovascular toxicity.

Serum paraoxonase (PON1) is a lipo-lactonase with antioxidant and atheroprotective properties. In many clinical and experimental studies, the atheroprotective effect of PON1 in protection against atherosclerosis has been proven. With its atheroprotective property, the PON1 enzyme can protect LDL and HDL from oxidation, reduce macrophage oxidative state, stimulate cholesterol efflux from macrophages and reduce oxidative state in atherosclerotic lesions. Atherosclerosis is the main cause of cardiovascular disease (CVD). PON1 activity is reduced in people with cardiovascular disease[28]. In addition, PON1 activity was found to be lower in patients with breast, lung, pancreatic, stomach, gastroesophageal and prostate cancers[29].

Enzymes are suitable targets for chemotherapeutic application in cancer therapy. Drug molecules bind to enzyme active sites. They exert their oncological effects by inhibiting the active site of the enzyme. There are few studies investigating the relationship between PON1 and anti-cancer drugs. These drugs showed an inhibitory effect on the PON1 enzyme. For example, in a study by Alım and Beydemir[26], the IC_{50} values of some anticancer drugs were in the range of 0.011-23.3 mM. Alım *et al.*[30] studied the *in vitro* inhibition effects of indazoles on PON1. They found that the IC_{50} values were in the range of 72.9-358 µM. In another study, Beydemir and Demir[27] investigated the effect of epilepsy medications on paraoxonase enzyme activity. They found that all drugs showed inhibitory effects. Isgör and Beydemir[31] studied the effects of some cardiovascular drugs on human serum PON1 enzyme activity. The IC_{50} values for these drugs were determined as 0.012 µM, 0.621 µM, 0.672 µM, 1.462 µM, 3.255 µM, 4.495 µM and 47.803 µM, respectively. A similar study was carried out by Türkes and coworkers. They investigated the *in vitro* effects of certain calcium channel blockers on purified human serum PON1. The IC_{50} values for

these drugs were determined as 0.121 mM, 0.130, 0.255 mM and 0.304 mM, respectively[25]. In my previous studies, the inhibitory effects of busulfan and carfilzomib on human serum PON1 were evaluated. The IC_{50} values were found to be 77 µM and 43.31 µM, respectively[32,33]. In our other works, the inhibitory effect of zoledronic acid, bendamustine, cladribine, mitoxantrone and methotrexate on PON1 were investigated and they inhibited PON1. Their IC_{50} values were 57 µM, 57 µM, 77 µM, 99 µM and 38.50 µM, respectively[34-37]. There has been no information about the inhibition types of trastuzumab and nivolumab on PON1 in the literature so far. Compared with other studies, these oncological drugs (trastuzumab and nivolumab) inhibited PON1 more potently than other drugs. However, this study should be supported by oncological studies.

CONCLUSION

As a result, because of the cardiovascular toxicity of oncological drugs (trastuzumab and nivolumab) used in cancer chemotherapy, the use of these drugs in cardiovascular patients may cause serious discomfort. Because decreases in PON1 activity in these patients may further increase Cardiovascular Diseases (CVD).

SIGNIFICANCE STATEMENT

This study discovered the inhibitory effect of *in vitro* oncological drugs (trastuzumab and nivolumab) on PON1, which no researchers have studied so far. Thus, a new theory can be obtained based on this study on how oncological drugs (trastuzumab and nivolumab) can increase cardiovascular diseases.

REFERENCES

1. Schirrmacher, V., 2019. From chemotherapy to biological therapy: A review of novel concepts to reduce the side effects of systemic cancer treatment (Review). Int. J. Oncol., 54: 407-419.
2. Bukowski, K., M. Kciuk and R. Kontek, 2020. Mechanisms of multidrug resistance in cancer chemotherapy. Int. J. Mol. Sci., Vol. 21. 10.3390/ijms21093233.
3. Scott, J.M., L.W. Jones, W.E. Hornsby, G.J. Koelwyn and M.G. Khouri *et al*., 2013. Cancer therapy-induced autonomic dysfunction in early breast cancer: Implications for aerobic exercise training. Int. J. Cardiol., 171: E50-E51.
4. Pugliatti, P., R. Donato, G. di Bella, S. Carerj and S. Patanè, 2014. Contrast-enhancing right atrial thrombus in cancer patient. Int. J. Cardiol., 173: E35-E37.
5. Kazory, A. and C. Ronco, 2019. Are we barking up the wrong tree? Rise in serum creatinine and heart failure. Blood Purif., 48: 193-195.
6. Calvagna, G.M. and S. Patanè, 2014. Transvenous pacemaker lead extraction in infective endocarditis. Int. J. Cardiol., 176: 511-513.
7. Lenihan, D.J., 2014. Cardiac biomarkers, cardiotoxicity, and active collaboration. J. Am. Coll. Cardiol., 63: 817-818.
8. Slamon, D., W. Eiermann, N. Robert, T. Pienkowski and M. Martin *et al*., 2011. Adjuvant trastuzumab in HER2-positive breast cancer. N. Engl. J. Med., 365: 1273-1283.
9. Fried, G., T. Regev and M. Moskovitz, 2013. Trastuzumab-related cardiac events in the treatment of early breast cancer. Breast Cancer Res. Treat., 142: 1-7.
10. Sandoo, A., G.D. Kitas and A.R. Carmichael, 2014. Endothelial dysfunction as a determinant of trastuzumab-mediated cardiotoxicity in patients with breast cancer. Anticancer Res., 34: 1147-1151.
11. Ferlay, J., I. Soerjomataram, R. Dikshit, S. Eser and C. Mathers *et al*., 2015. Cancer incidence and mortality worldwide: Sources, methods and major patterns in GLOBOCAN 2012. Int. J. Cancer, 136: E359-E386.
12. Global Burden of Disease Cancer Collaboration, 2018. Global, regional, and national cancer incidence, mortality, years of life lost, years lived with disability, and disability-adjusted life-years for 29 cancer groups, 1990 to 2016: A systematic analysis for the global burden of disease study. JAMA Oncol., 4: 1553-1568.
13. Banke, A., E.L. Fosbøl, M. Ewertz, L. Videbæk and J.S. Dahl *et al*., 2019. Long-term risk of heart failure in breast cancer patients after adjuvant chemotherapy with or without trastuzumab. JACC: Heart Fail., 7: 217-224.
14. Koppolu, V. and V.K.R. Vasigala, 2018. Checkpoint immunotherapy by nivolumab for treatment of metastatic melanoma. J. Cancer Res. Ther., 14: 1167-1175.
15. Lim, J.S.J. and R.A. Soo, 2016. Nivolumab in the treatment of metastatic squamous non-small cell lung cancer: A review of the evidence. Ther. Adv. Respir. Dis., 10: 444-454.
16. Mozaffarian, D., E.J. Benjamin, A.S. Go, D.K. Arnett and M.J. Blaha *et al*., 2016. Heart disease and stroke statistics-2016 update: A report from the American Heart Association. Circulation, 133: e38-e360.
17. Yu, A.F., N.U. Yadav, B.Y. Lung, A.A. Eaton and H.T. Thaler *et al*., 2015. Trastuzumab interruption and treatment-induced cardiotoxicity in early HER2-positive breast cancer. Breast Cancer Res. Treat., 149: 489-495.
18. Herrmann, J., 2020. Adverse cardiac effects of cancer therapies: Cardiotoxicity and arrhythmia. Nat. Rev. Cardiol., 17: 474-502.
19. Shuvalov, O., A. Petukhov, A. Daks, O. Fedorova, E. Vasileva and N.A. Barlev, 2017. One-carbon metabolism and nucleotide biosynthesis as attractive targets for anticancer therapy. Oncotarget, 8: 23955-23977.

20. Bizoń, A. and H. Milnerowicz, 2018. The effect of divalent metal chelators and cadmium on serum phosphotriesterase, lactonase and arylesterase activities of paraoxonase 1. Environ. Toxicol. Pharmacol., 58: 77-83.
21. Faisal, A.J., L.A. Said and M.R. Ali, 2021. Quorum quenching effect of recombinant Paraoxonase-1 enzyme against quorum sensing genes produced from *Pseudomonas aeruginosa*. Gene Rep., Vol. 25. 10.1016/j.genrep.2021.101412.
22. Mahrooz, A., 2016. Pharmacological interactions of paraoxonase 1 (PON1): A HDL-bound antiatherogenic enzyme. Curr. Clin. Pharmacol., 11: 259-264.
23. Chistiakov, D.A., A.A. Melnichenko, A.N. Orekhov and Y.V. Bobryshev, 2017. Paraoxonase and atherosclerosis-related cardiovascular diseases. Biochimie, 132: 19-27.
24. Arenas, M., E. Rodríguez, A. Sahebkar, S. Sabater and D. Rizo *et al*., 2018. Paraoxonase-1 activity in patients with cancer: A systematic review and meta-analysis. Crit. Rev. Oncol./Hematol., 127: 6-14.
25. Türkeş, C., H. Söyüt and Ş. Beydemir, 2014. Effect of calcium channel blockers on paraoxonase-1 (PON1) activity and oxidative stress. Pharmacol. Rep., 66: 74-80.
26. Alim, Z. and Ş. Beydemir, 2016. Some anticancer agents act on human serum paraoxonase-1 to reduce its activity. Chem. Biol. Drug Des., 88: 188-196.
27. Beydemir, Ş. and Y. Demir, 2017. Antiepileptic drugs: Impacts on human serum paraoxonase-1. J. Biochem. Mol. Toxicol., Vol. 31. 10.1002/jbt.21889.
28. Aharoni, S., M. Aviram and B. Fuhrman, 2013. Paraoxonase 1 (PON1) reduces macrophage inflammatory responses. Atherosclerosis, 228: 353-361.
29. Bacchetti, T., G. Ferretti and A. Sahebkar, 2019. The role of paraoxonase in cancer. Semin. Cancer Biol., 56: 72-86.
30. Alım, Z., D. Kılıç and Y. Demir, 2019. Some indazoles reduced the activity of human serum paraoxonase 1, an antioxidant enzyme: *in vitro* inhibition and molecular modeling studies. Arch. Physiol. Biochem., 125: 387-395.
31. Isgor, M.M. and S. Beydemir, 2010. Some cardiovascular therapeutics inhibit paraoxonase 1 (PON1) from human serum. Eur. J. Pharmacol., 645: 135-142.
32. Soyut, H., 2021. Investigation of inhibition of busulfan (chemotherapeutic drug) on human serum paraoxonase-1 (PON1). Int. J. Pharmacol., 17: 572-576.
33. Soyut, H., 2022. An *in vitro* study: Inhibitory effect of carfilzomib on human serum paraoxonase-1 (hPON1). Int. J. Pharmacol., 18: 522-526.
34. Söyüt, H., Y. Ulutaş and E. Köksal, 2020. Inhibitory effects of bendamustine on human serum paraoxonase-1 (hPON1). J. Inst. Sci. Technol., 10: 1833-1838.
35. Söyüt, H., Y. Ulutaş and E. Köksal, 2020. Investigation of *in vitro* inhibition effect of zoledronic acid on human serum paraoxonase1 (PON1) enzyme activity. Turk. J. Nat. Sci., 9: 185-189.
36. Söyüt, H., Y. Ulutaş and E. Köksal, 2022. Investigation of inhibition effect of some chemotherapeutic drugs on human serum paraoxonase-1 (PON1). J. Inst. Sci. Technol., 12: 297-305.
37. Söyüt, H., Y. Ulutaş and E. Köksal, 2022. Inhibitory effect of methotrexate (MTX) used in human cancer treatment on paraoxonase-1 (PON1). Int. J. Pharmacol., 18: 942-946.