**In Vitro Effects of Some Chemotherapy Drugs on Glucose-6-Phosphate Dehydrogenase Enzyme Purified from Sheep Spleen**

*Çiğdem ÇOBAN1,0000-0003-1141-544X , Mehmet ÇİFTCİ2,\*,0000-0002-1748-3729*

*1Bingol University,*  [*Solhan Health Services Vocational School*](https://batman.edu.tr/Birimler/1472)*, Bingol, Türkiye*

*2Bingol University,* [*Faculty*](http://sshmyo.bingol.edu.tr/en/) *of Veterinary Science,Bingol, Türkiye*

|  |
| --- |
| **Abstract**  In this study, the inhibition effects of some important drugs used in chemotherapy on glucose-6-phosphate dehydrogenase, a NADP+-dependent enzyme obtained from sheep spleen tissue, were examined in vitro. Sheep spleen glucose-6-phosphate dehydrogenase enzyme (D-glucose-6-phosphate: NADP+ oxidoreductase, EC 1.1.1.49, G6PD) was first purified by 2', 5'-ADP Sepharose 4B affinity chromatography. Enzyme activity was determined spectrophotometrically at 340 nm by the Beutler method. This method was applied in all kinetic studies. Carboplatin, cisplatin, oxaloplatin, fluorouracil, cyclophosphamide and ibandronic acid were used as drugs. In vitro studies showed that oxaloplatin, carboplatin and cyclophosphamide drugs had inhibitory effects on the enzyme in question. It was observed that other drugs did not affect the enzyme much. IC50 values were found by drawing % Activity-[I] graphs for drugs showing inhibitory effects. The IC50 values obtained for oxaloplatin, carboplatin and cyclophosphamide drugs were 3.22 mM, 7.26 mM, and 34.5 mM, respectively. |
| ***Key Words:*** *Glucose-6-phosphate dehydrogenase, Kemoterapi ilaçları, Inhibiton* |

1. **Introduction**

Glucose 6-phosphate dehydrogenase (G6PD) (E.C. 1.1.1.49) catalyzes the first reaction of the pentose phosphate metabolic pathway. The only source for NADPH formation in erythrocytes is the pentose phosphate metabolic pathway, and NADPH is significantly reduced in G6PD deficiency. The most important role of NADPH in erythrocytes is to reduce oxidized glutathione (GSH). This reaction is catalyzed by glutathione reductase. The reduced form of glutathione (GSSH) is a tripeptide containing a free thiol group. The free thiol group acts as a sulfhydryl buffer, keeping hemoglobin and erythrocyte proteins in a reduced state; It also takes part in detoxification events by reacting with hydrogen peroxide and organic peroxides. For this reason, G6PD can be defined as an indirect antioxidant enzyme. As a result, this enzyme is for living things; It is very important in growth, development and protection against oxidative stress. NADPH is also a coenzyme involved in the synthesis of biomolecules such as fatty acids, steroids and some amino acids. Many drugs such as anti-inflammatory antibiotics and anesthetics are used in the medical treatment of animal diseases. However, little information is available about the effects of drugs on different enzymes. For example, the in vivo and in vitro inhibition effects of metamizole, amikacin sulfate, sodium ampicillin, and netilmicin sulfate drugs on G6PD enzyme activity obtained from rat erythrocytes were examined. In this study, the purification of G6PD enzyme from sheep spleen tissue and the effects of drugs such as carboplatin, cisplatin, oxaloplatin, fluorouracil, cyclophosphamide and ibandronic acid on this enzyme activity were investigated [1-3].

1. **Materials and Methods** 
   1. **Preparation of Hemogenate**

The spleen tissue, obtained from the Bingöl Provincial Combined Meat and Milk Institution in accordance with the cold chain rules, was first divided into certain pieces in the laboratory and stored at -20°C. During the experiment, 15 g of spleen tissue was taken, chopped into small pieces, and suspended in 45 mL of 50 mM KH2PO4 (pH 7.5) buffer. Then, homogenate was formed by centrifuging at 13,000xg for 1 hour and removing the precipitate [4-6].

* 1. **2', 5'-ADP Sepharose 4B Affinity Chromatography**

The dialyzed enzyme solution was loaded into the column and the flow rate was set to 20 mL/hour. Then, the column was injected successively with 25 mL 0.1 M K-acetate + 0.1 M K-phosphate, (pH: 6.0) and 25 mL 0.1 M KCl + 0.1 M K-phosphate (pH: 7, 85) was washed with. Washing with 0.1 M KCl + 0.1 M K-phosphate (pH: 7.85) was continued until the absorbance difference became 0.05 at 280 nm. Finally, the enzyme was eluted with a solution of 80 mM K-phosphate + 80 mM KCl + 0.5 mM NADP + + 10 mM EDTA (pH: 7.85). Enzyme activities were measured in the fractions. Tubes showing activity were pooled together. Protein determination was made in the final solution. The temperature was kept at +4 oC throughout all procedures [4,7].

* 1. **Activity Determination**

Activity determination was made according to the Beutler method. An enzyme unit was defined as the amount of enzyme that converts 1 mol NADP+ into NADPH in 1 minute [4,8].

* 1. **Protein Determination**

Quantitative protein determination was performed spectrophotometrically at 595 nm according to the Bradford method, using bovine serum albumin as the standard [9].

* 1. **Kinetic Studies**

The effects of drugs such as carboplatin, cisplatin, oxaloplatin, fluorouracil, cyclophosphamide and ibandronic acid on G6PD were examined. The tube containing no drug was used as a control and its activity was accepted as 100%. For each drug that inhibits the enzyme, a Activity%-[Drug] graph was drawn at different inhibitor concentrations. IC50 values were calculated through these graphs.

1. **Results**

Activity%-[Drug] graphs drawn for drugs showing inhibitory effects on sheep spleen G6PD enzyme are shown in Figures 1, 2, and 3. The IC50 values obtained for the drugs oxaloplatin, carboplatin and cyclophosphamide, which have an inhibitory effect on the enzyme, were 3.22, 7.26, and 34.50 mM, respectively, and are shown in Table 1.

Figure 1. Activity%-[I] graph obtained for oxaloplatin

Figure 2. Activity%-[I] graph obtained for carboplatin

Figure 3. Activity%-[I] graph obtained for cyclophosphamide

Table 1. Obtained IC50 values

|  |  |
| --- | --- |
| Drugs | IC50 (mM) |
| Oxaliplatin | 3,22 |
| Carboplatin | 7,26 |
| Cyclophosphamide | 34,5 |

1. **Conclusion**

Because of its important functions in the pentose phosphate pathway, the G6PD enzyme has been purified and characterized from many human, animal and plant tissues. Especially recently, the effects of many medical drugs and different chemicals on enzyme activity have been investigated. Inhibition of G6PD is of vital importance, especially in patients with G6PD deficiency. In this study, the effects of drugs such as carboplatin, cisplatin, oxaloplatin, fluorouracil, cyclophosphamide and ibandronic acid on the enzyme were investigated. As a result of the research, although the drugs cisplatin, fluorouracil and ibandronic acid did not have significant effects on the enzyme, it was determined that oxaloplatin, carboplatin and cyclophosphamide drugs inhibited the enzyme to a significant extent, and %Activity-[Drug] graphs were drawn for these drugs (Figures 1, 2, 3). ) IC50 values were found (Table 1). According to these IC50 values (inhibitor concentration that reduces the enzyme activity by half), the decreasing inhibitory powers of the drugs can be listed as oxaloplatin, carboplatin and cyclophosphamide. Because while oxaloplatin with a concentration of 3.22 mM causes 50% inhibition, this value is 7.26 mM for carboplatin and 34.5 mM for cyclophosphamide. According to the IC50 values obtained in this study, it is understood that the strongest inhibitor is oxaloplatin, followed by carboplatin and cyclophosphamide.

As a result, it will be important to conduct more detailed inhibition studies for these three drugs in future studies, determine Ki constants and conduct *in vivo* studies. In addition, it will be important for body metabolism to take these IC50 values into consideration when adjusting the dosages of these drugs used in treatment.

**References**

1. Lehninger, AL., Nelson, DL., Cox, MM. (1993) *Principles of Biochemistry*, 2nd edn. New York: Worth Publishers Inc, 436-437.
2. Kanji, MI., Toews, ML., Carpar, WR. (1976) A kinetic study of glucose-6-phosphate dehydrogenase. *J Biol Chem*, 25, 2258-2262.
3. Yuregir, GT., Aksoy, K., Arpaci, A., Unlukurt, I., Tuli, A. (1994) Studies on red cell glucose-6-phosphate dehydrogenase, evaluation of reference values. *Ann Clin Biochem*, 31, 50-55.
4. Ninfali, P., Orsenigo, T., Barociani, L., Rapa, S. (1990) Rapid purification of glucose-6-phosphate dehydrogenase from mammal’s erythrocytes. *Prep Biochem*, 20, 297-309.
5. Delgado, C., Tejedor, C., Luquue, J. (1990) Partial purification of glucose-6-phosphate dehydrogenase and phosphofructokinase from rat erythrocyte haemolysate by partitioning in aqueous two-phase systems. *J Chromatographi*, 498, 159-168.
6. Shreve, DS., Levy, HR. (1977) On the molecular weight of human glucose-6-phosphate dehydrogenase. *Biochem Biophy Res Com*, 78, 1369-1375.
7. Morelli, A., Benatti, U., Gaetani, GF., De Flora, A. (1979) Biochemical mechanisms of glucose-6-phosphate dehydrogenase deficiency. *Proc Natl Acad Sci*, 75, 1979-1983.
8. Beutler, E. (1971) *Red Cell Metabolism Manual of Biochemical Methods*. London: Academic Press, 68-70.
9. Bradford MM. (1976) A rapid and sensitive method for the quantition of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem*, 72, 248-251