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Effects of some drugs on hepatic glucose 6-phosphate dehydrogenase activity in Lake Van Fish (*Chalcalburnus Tarischii* Pallas, 1811)

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Abstract

Inhibitory effects of some drugs on hepatic glucose 6-phosphate dehydrogenase from Lake Van fish (*chalcalburnus tarischii* pallas, 1811) were investigated. For this purpose, initially liver glucose 6-phosphate dehydrogenase was purified 899-fold in a yield of 46.24% by using 2′,5′-ADP Sepharose 4B affinity gel. In order to control the purification of enzyme was done SDS polyacrylamide gel electrophoresis. SDS polyacrylamide gel electrophoresis showed a single band for enzyme. A constant temperature (+4 °C) was maintained during the purification process. Enzyme activity was determined with the Beutler method by using a spectrophotometer at 340 nm.

Vankomycine, sulfanylamide, sulfanylacetamide, nidazole, ciprofloxacin, amoxicillin and $KMnO_4$ were used as drugs. These drugs exhibited inhibitory effects on the enzyme.

IC_{50} values of vankomycine, sulfanylamide, sulfanylacetamide, nidazole, ciprofloxacin, amoxicillin and $KMnO_4$ were 1.88, 0.037, 0.032, 1.178, 2.26, 643.5 and 0.0002 mM, and the K_i constants 1.18 ± 0.148, 0.119 ± 0.021, 0.075 ± 0.015, 1.15 ± 0.21, 7.69 ± 0.67, 1007 ± 69, and 0.001 ± 0.00022 mM, respectively. While vankomycine and nidazole showed competitive inhibition, others displayed noncompetitive inhibition. K_i constants and IC_{50} values for drugs were determined by Lineweaver–Burk graphs and plotting activity percentage versus [*I*], respectively.

Keywords: G6PD Enzyme; *Chalcalburnus Tarischii*; Inhibition; Drug

1. Introduction

Glucose 6-phosphate dehydrogenase (E.C.1.1.49; G6PD) is the key and first enzyme of the pentose phosphate metabolic pathway, catalyzing the conversion of glucose 6-phosphate to 6-phosphogluconate in the presence of $NADP^+$. The main physiological function of G6PD is to produce NADPH and ribose 5-phosphate. NADPH and ribose 5-phosphate are essential for reductive biosynthesis, nucleic acid and membrane lipids synthesis [1–3]. $NADP^+$ is reduced to NADPH by glutathione reductase in erythrocytes using glutathione as substrate. Glutathione prevents hemoglobin denaturation, preserves the integrity of red blood cell membrane sulfhydryl groups, and detoxifies hydrogen peroxide and oxygen radicals in the red blood cells [4,5]. A decrease in G6PD may result in NADPH and reduced glutathione deficiency in erythrocytes; scarcity of reduced glutathione in erythrocytes causes early haemolysis in spleen [6].

When one molecule palmitate is synthesized in the biosynthesis of fatty acids, 14 NADPH molecules are used. Six of these 14 NADPH molecules come from the pentose phosphate pathway. Essentially, the pentose phosphate pathway is more active in adipose tissues compared with muscles, showing that the G6PD enzyme is very important in the biosynthesis of fatty acids [3,7]. NADPH plays an important role in initiating protein synthesis [8]. Fish in general require more dietary protein than do other vertebrates. NADPH is essential to the growth and proliferation processes, serving as hydrogen and electron sources for a variety of reductive biosynthetic reactions, including the synthesis of fatty acids and cholesterol [9].

The effects of many drugs on rainbow trout, human and rat G6PD enzyme activities have been investigated [10–13]. However, no reports could be found in the literature on the effects of vankomycine, sulfanylamide, sulfanylacetamide, nidazole,

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ciprofloxacin, amoxicillin and $KMnO_4$ on Lake Van fish liver G6PD.

Vankomycine, sulfanylamide, sulfanylacetamide, nidazole, ciprofloxacin, amoxicillin have been key players against bacterial pathogens [14]. $KMnO_4$ is used for Larnea sickness. All drugs above are used in fish sickness.

The aim of this study was purifying Lake Van fish (*Chalcalburnus tarischii*) liver G6PD and (The pH of Lake Van is 9.5 and the depth is 450 m) determination of inhibition or activation effects of some drugs on Lake Van fish hepatic G6PD activities were investigated.

2. Materials and methods

2.1. Materials

2′,5′-ADP Sepharose 4B was purchased from Pharmacia. $NADP^+$, glucose 6-phosphate, protein assay reagent, and chemicals for electrophoresis were purchased from Sigma Chem. Co. All other chemicals were analytical reagent grade and purchased from either Sigma or Merck.

2.2. Preparation of the homogenate

Wild Lake Van fish samples were obtained from Lake Van (pH 9.5; depth, 450 m) (Van, Turkey). The Lake Van Fish ($n = 5$) used in this study were mature, healthy, adult fish with an average weight of 80–100 g.

Fresh Lake Van fish liver (8 g) was cut with a knife. Excess blood, foreign tissues and membranes were removed from the samples. Tissue was suspended in 100 ml of 5 mM phosphate buffer (pH 7.4) containing 458 mM saccharose, and was homogenized using a mixer at top speed for 3 min. Then, the material was homogenized by ultrasonic homogenizer for 5 min. After that, the homogenate was centrifuged at 12,100 rpm (21,200 × *g*) for 60 min, and the supernatant was removed. This process was repeated three times and temperature was maintained at 4 °C during the homogenization process [15].

2.3. 2′,5′-ADP sepharose 4B affinity chromatography

2′,5′-ADP Sepharose 4B Affinity Chromatography was done according to Ninfali et al. [16], Morelli et al. [17] and Muto and Tan [18].

2.4. Activity determination and optimal pH determination

The enzymatic activity was measured by Beutler's method [19]. One enzyme unit was defined as the enzyme amount reducing 1 μmol $NADP^+$ per minute.

2.5. Protein determination

Quantitative protein determination was spectrophotometrically measured at 595 nm according to Bradford's method [20], with bovine serum albumin being used as a standard.

2.6. SDS polyacrylamide gel electrophoresis (SDS-PAGE)

Enzyme purity was determined using Laemmli's procedure [21], and was carried out in 3% and 8% acrylamide concentrations for stacking and running gel, respectively.

2.7. Inhibition studies

In order to determine IC_{50} values, activities were calculated with a 0.60 mM substrate (G6P), 25 °C, optimum pH (pH 8.5; 0.1 M Tris–HCl) and different inhibitor concentrations. Drugless cuvette activity was taken as 100%. The inhibitor concentrations causing 50% inhibition (IC_{50}) were determined from the graphs.

In order to determine K_i constants in the media with or without inhibitor, the substrate (G6P) concentrations were 0.06, 0.12, 0.18, 0.24, and 0.30 mM. Inhibitors (drugs) solutions were added to the reaction medium, resulting in three different fixed concentrations of inhibitors in 1 ml of total reaction volume. Lineweaver–Burk graphs [22] were drawn by using $1/V$ versus $1/[S]$ values and K_i constant were calculated from these graphs. Regression analysis graphs were drawn using inhibition percentage values by a statistical package (SPSS-for windows; version 10.0) on a computer (Student's *t*-test; $n = 3$).

3. Results

G6PD was purified 899-fold in a yield of 46.24% by using 2′,5′-ADP Sepharose 4B affinity gel (Table 1). SDS polyacrylamide gel electrophoresis was performed after the purification of the enzyme, and the electrophoretic pattern was photographed (Fig. 1). Optimal pH of G6PD has been found as 8.5 in 0.1 M Tris–HCl.

IC_{50} values of vankomycine, sulfanylamide, sulfanylacetamide, nidazole, ciprofloxacin, amoxicillin and $KMnO_4$ were 1.88, 0.037, 0.032, 1.178, 2.26, 643.5 and 2.10^{-4} mM, and

Table 1
Purification scheme of glucose-6-phosphate dehydrogenase from Lake Van fish liver

Purification step	Activity (U/ml)	Total volume (ml)	Protein (mg/ml)	Total protein (mg)	Total activity (U)	Specific activity (U/mg)	Yield (%)	Purification factor
Homogenate	4.75	28	124	3.472	133	0.038	100	1
2′,5′-ADP sepharose 4B affinity chromatography	4.1	15	0.12	1.8	61.5	34.16	46.24	899

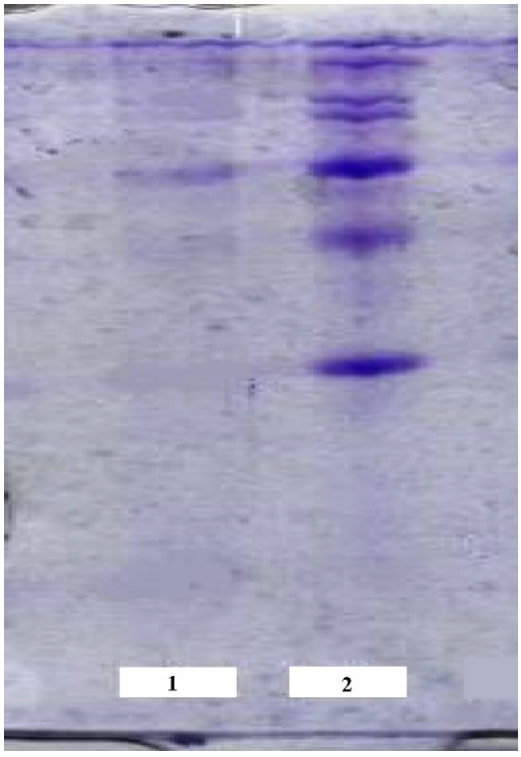

Fig. 1. SDS-PAGE bands of G6PD (Lane 2; standards: rabbit myosin (205,000), *E. coli* β-galactosidase (116,000), rabbit phosphorylase B (97,400), bovine albumin (66,000), chicken ovalbumin (45,000), and bovine carbonic anhydrase (29,000)). Lane 1: Lake Van Fish liver G6PD.

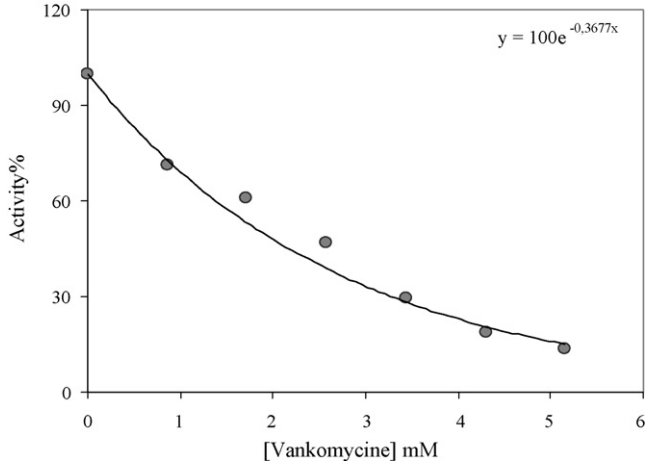

Fig. 2. Activity percentage vs. [vankomycine] regression analysis graphs for Lake Van Fish liver G6PD in the presence of five different vankomycine concentrations.

the K_i constants 1.18 ± 0.148, 0.119 ± 0.021, 0.075 ± 0.015, 1.15 ± 0.21, 7.69 ± 0.67, 1007 ± 69, and 0.001 ± 0.00022 mM, respectively (Figs. 2 and 3).

4. Discussion

It is known that many drugs have adverse effects on the organism when used for therapeutic or other purposes [23]. These effects may be dramatic and systematic [10]. A good example of this is that in 1926 pamaquine used for malaria treatment caused severe adverse effects in patients within a few days, resulting in black urination, hyperbilirubinemia, a dramatic decrease in blood Hb levels, and finally death, which occurred in cases of severe G6PD deficiency [7]. Similarly, acetazolamide inhibits carbonic anhydrase (CA) resulting in with severe diuresis [24]. Therefore, investigation of the effects of some drugs on the enzyme activity of glucose 6-phosphate dehydrogenase from Lake Van fish liver is very important.

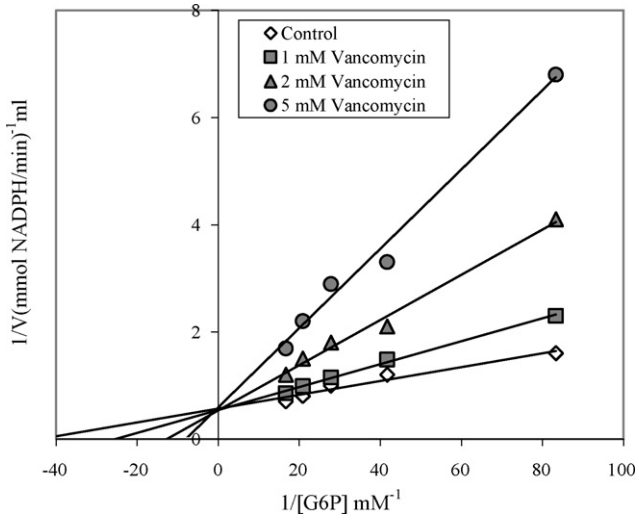

Fig. 3. Lineweaver–Burk graph in five different substrate (G6P) concentrations and in three different vankomycine concentrations for determination of K_i.

Lake Van fish liver G6PD enzyme was purified with a 46.24 of yield and 899-fold in 5 or 6 h by using 2′,5′-ADP Sepharose 4B affinity gel chromatography (Table 1) in this study. Fig. 1 exhibits the SDS-PAGE done for the determination of purity of the enzyme. A high purity for the enzyme has been obtained.

Inhibitory effects of many drugs on G6PD enzyme activities in different animal species and human beings have been reported in many investigations [10,11,25]. For example, it has been reported that thiamphenicol, amikacin, gentamicin, netilmicin, chloramine-T and $CuSO_4$ inhibit rainbow trout erythrocyte G6PD [12,13]. Effects of many drugs such as antibiotics, analgesic and anesthetic were investigated on human erythrocytes G6PD [26], sheep erythrocyte G6PD [25] and sheep liver G6PD [27].

However, to the best of our knowledge, the inhibitory effects of these drugs on G6PD in Lake Van fish liver have not been studied.

In order to show inhibitory effects, while the most suitable parameter is the K_i constant, some researchers use the IC_{50} value. Therefore, in this study, both the K_i and IC_{50} parameters of these drugs for G6PD were determined.

IC_{50} values of $KMnO_4$, sulfanylacetamide, sulfanilamide, nidazole, vankomycine, ciprofloxacin and amoxicillin were 0.0002, 0.032, 0.037, 1.178, 1.88, 2.26, and 643.5 mM, and the K_i constants were 0.001 ± 0.00022, 0.075 ± 0.015, 0.119 ± 0.021, 1.15 ± 0.21, 1.18 ± 0.148, 7.69 ± 0.67, and 1007 ± 69 mM, respectively. K_i values show that $KMnO_4$ had the highest inhibitor effect, followed by sulfanylacetamide,

Table 2
K_i and I_{50} values obtained from regression analysis graphs for G6PD in the presence of different drugs concentrations

Drugs	K_i (mM)	IC_{50} (mM)
$KMnO_4$	0.001 ± 0.00022	0.0002
Sulfanylacetamide	0.075 ± 0.015	0.032
Sulfanylamide	0.119 ± 0.021	0.037
Nidazole	1.15 ± 0.21	1.178
Vankomycine	1.18 ± 0.148	1.88
Ciprofloxacin	7.69 ± 0.67	2.26
Amoxicillin	1007 ± 69	643

sulfanilamide, nidazole, vankomycine, ciprofloxacin and amoxicillin, respectively. IC_{50} values showed the same trend (Table 2).

Inhibitory effect of vankomycine on human erythrocyte G6PD (IC_{50}: 0.903 mM and K_i: 2.7 mM) [28], rainbow trout erythrocyte G6PD (IC_{50}: 4.789 mM and K_i: 1.466 mM) [29], and sheep lens G6PD (IC_{50}: 8 mM) [30] were reported, suggesting higher affinity of Lake Van fish liver G6PD to vankomycine compared with sheep lens and rainbow trout erythrocyte G6PD.

In this investigation, these drugs showed highly inhibitory effects on the G6PD enzyme activity of Lake Van fish liver. By using the obtained K_i and IC_{50} values, undesirable side effects of these drugs on G6PD activity and body metabolism and fatty acid synthesis can be reduced. The dosages of drugs used in fishery farms as follows: vankomycine ∼0.0267, sulfanylamide ∼0.64, sulfanylacetamide ∼0.5, nidazole ∼0.0234, ciprofloxacin ∼0.02, amoxicillin ∼0.028 and $KMnO_4$ ∼0.063 mM [14]. By taking into these concentrations account, the inhibition data calculated from plots were found to be 1, 100, 100, 1, 1, 1 and 100%, respectively. According to these data, sulfanylamide, sulfanylacetamide and $KMnO_4$ should not be used in fishery farms, but other drugs may be used quite carefully.

Besides, today in the world, the number of fish farmers is rising and a lot of antibiotics and drugs are used in the treatment of fish diseases. The fish eaters take the metabolic products of these drugs with nutrient chain. These metabolic products may have side effects. If it is required to give to fish, half-lives of drugs and drug metabolites must also be taken into consideration.

References

[1] R.B. Pilz, R.C. Willis, G.R. Boss, The influence of ribose-5-phosphate availability on purine synthesis of cultured human lymphoblasts and mitogen-stimulated lymphocytes, J. Biol. Chem. 259 (1984) 2927–2935.

[2] W. Kuo, J. Lin, T.K. Tang, Human glucose-6-phosphate dehydrogenase (G6PD) gene transforms nih 3t3 cells and induces tumors in nude mice, Int. J. Cancer. 85 (2000) 857–864.

[3] A.L. Lehninger, D.L. Nelson, M.M. Cox, Principles of Biochemistry, 2nd ed., Worth Publishers Inc., New York, 2000, pp. 558–560.

[4] J. Deutsch, Glucose-6-phosphate dehydrogenase, in: H.U. Bergmeyer, J. Bergmeyer (Eds.), Methods of Enzymatic Analysis, VCH, Verlagsgerellschaff, 1990, pp. 190–196.

[5] B.B. Weksler, A. Moore, J. Tepler, in: T.E. Andreoli, C.C.J. Carpenter, F. Plum, L.H. Smith Jr. (Eds.), Hematology, Cecil Essentials of Medicine, 2nd ed., WB Saunders Company, Philadelphia, 1990, pp. 341–363.

[6] M.M. Andrews, K.H. Money, Alterations in haematologic function in children, in: K.L. McCance, S.E. Huether (Eds.), Pathophysiology. The Biologic Basis for Disease in Adults and Children, 2nd ed., Mosby Year Book Inc., USA, 1994, pp. 908–942.

[7] E.E. Keha, O.I. Kufrevioglu, Biyokimya (Turkish), 2nd ed., Aktif Yayinevi, Istanbul, 2000, pp. 356–366.

[8] B. Kan, I.M. London, D.H. Levin, Role of reversing factor in the inhibition of protein synthesis initiation by oxidized gluthatione, J. Biol. Chem. 263 (1988) 15652–15656.

[9] K. Ogawa, D.B. Solt, E. Farber, Phenotypic diversity as an early property of putative preneoplastic hepatocyte populations in liver carcinogenesis, Cancer Res. 40 (1980) 725–733.

[10] M. Ciftci, O.I. Kufrevioglu, M. Gundogdu, I. Ozmen, Effects of some antibiotics on enzyme activity of glucose-6-phosphate dehydrogenase from human erythrocytes, Pharmacol Res. 41 (2000) 109–113.

[11] M. Ciftci, I. Ozmen, M.E. Buyukokuroglu, S. Pence, O.I. Kufrevioglu, Effects of metamizol and magnesium sulfate on enzyme activity of glucose-6-phosphate dehydrogenase from human erythrocytes *in vitro* and rat erythrocyte *in vivo*, Clin. Biochem. 34 (2001) 297–302.

[12] A. Ciltas, O. Erdogan, O. Hisar, M. Ciftci, Effects of chloramine-*t* and $CuSO_4$ on enzyme activity of glucose 6-phosphate dehydrogenase from rainbow trout *(Oncorhynchus mykiss)* erythrocytes *in vitro* and *in vivo*, Isr. J. Aquacult.–Bamid. 55 (3) (2003) 187–196.

[13] O. Erdogan, M. Ciftci, A. Çiltas, O. Hisar, Inhibition effects of some antibiotics on the activity of glucose 6-phosphate dehydrogenase enzyme from rainbow trout *(Oncorhynchus mykiss* walbaum, 1792) erythrocytes, Turk. J. Vet. Anim. Sci. 28 (2004) 675–681.

[14] S.O. Kayaalp, Rasyonal tedavi yönünden tıbbi farmakoloji. 2002. Hacettepe-Tas Yayıncılık, Ankara (Turkish).

[15] I. Mohammed, M.L. Tuews, W.R. Carpar, A kinetic study of glucose 6-phosphate dehydrogenase, J. Biol. Chem. 251 (8) (1976) 2255–2257.

[16] P. Ninfali, T. Orsenigo, L. Barociani, S. Rapa, Rapid purification of glucose-6 phosphate dehydrogenase from mammal's erythrocyte, Prep. Biochem. 20 (1990) 297–309.

[17] A. Morelli, U. Benatti, G.F. Gaetani, A. De Flora, Biochemical mechanisms of glucose-6-phosphate dehydrogenase deficiency, Proc. Natl. Acad. Sci. U.S.A. 75 (1978) 1979–1983.

[18] N. Muto, L. Tan, Purification of oestrogen synthesase by high performance liquid chromatography. Two membrane bound enzymes from the human placenta, J. Chromatogr. 326 (1985) 137–146.

[19] E. Beutler, Red Cell Metabolism Manual of Biochemical Methods, Academic Press, London, 1971, pp. 68–70.

[20] M.M. Bradford, A rapid and sensitive method for the quantization of microgram quantities of protein utilizing the principle of protein-dye binding, Anal. Biochem. 72 (1976) 248–251.

[21] U.K. Laemmli, Clevage of structural proteins during assembly of the head of bacteriophage, T_4 Nature 227 (1970) 680–683.

[22] I.E. Segel, Enzyme Kinetics, John Wiley and Sons, Toronto, 1975.

[23] R.M. Hochster, M. Kates, J.H. Quastel, Metabolic Inhibitors, Academic Press, New York, 1972, pp. 71–89.

[24] D.G. Warnock, Diuretic Agent, in: B.G. Katzung (Ed.), Basic and Clinical Pharmacology, 4th ed., Appleton and Lange, USA, 1989, pp. 183–197.

[25] S. Beydemir, M. Ciftci, O.I. Kufrevioglu, Purification and characterization of glucose 6-phosphate dehydrogenase from sheep erythrocytes, and inhibitory effects of some antibiotics on enzyme activity, J. Enzyme Inhib. Med. Chem. 17 (4) (2002) 271–277.

[26] S. Altikat, M. Ciftci, M.E. Büyükokuroglu, *In vitro* effects of some anesthetic drugs on enzymatic activity of human red blood cell glucose 6-phosphate dehydrogenase, Pol. J. Pharmacol. 54 (2002) 67–71.

[27] V. Turkoglu, S. Aldemir, M. Ciftci, Purification and characterization of glucose 6-phosphate dehydrogenase from sheep liver, Turk. J. Chem. 27 (2003) 395–402.

[28] H. Ozdemir, M. Ciftci, In vitro effects of some drugs on human red blood cell glucose-6-phosphate dehydrogenase enzyme activity, J. Enzyme Inhib. Med. Chem. 21 (1) (2006) 75–80.

[29] M. Ciftci, Effects of some drugs on the activity of glucose 6-phosphate dehydrogenase from rainbow trout (*Oncorhynchus mykiss*) erythrocytes in vitro, J. Enzyme Inhib. Med. Chem. 20 (5) (2005) 485–489.

[30] S. Beydemir, D.N. Kulacoğlu, M. Ciftci, O.I. Kufrevioglu, The effects of some antibiotics on sheep lens glucose 6-phosphate dehydrogenase *in vitro*, Eur. J. Ophthalmol. 13 (2) (2003) 155–161.