Effects of some antibiotics on glucose 6-phosphate dehydrogenase in sheep liver

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ABSTRACT: *In vitro* effects of penicillin, sulbactam, cefazolin, and amikacine on the activity of the enzyme glucose-6-phosphate dehydrogenase in sheep liver were investigated. Glucose 6-phosphate dehydrogenase was purified from sheep liver, using a simple and rapid method. The purification consisted of two steps, preparation of homogenate and 2', 5'-ADP Sepharose 4B affinity chromatography. As a result of the two consecutive procedures, the enzyme, having the specific activity of 11.76 EU/mg proteins, was purified with a yield of 35.72% and 1.913 fold. In order to control the enzyme purification SDS polyacrylamide gel electrophoresis (SDS-PAGE) was done. SDS-PAGE showed a single band for the enzyme. In addition, I_{50} values of the antibiotics were determined by plotting activity % vs. antibiotic concentrations. I_{50} values were 17.71 mM for penicillin, 27.38 mM for sulbactam, 28.88 mM for cefazolin, and 30.59 mM for amikacine.

Keywords: glucose 6-phosphate dehydrogenase; antibiotics; sheep; liver

Glucose 6-phosphate dehydrogenase (D-glucose 6-phosphate: $NADP^+$ oxidoreductase EC 1.1.1.49; G6PD) is the first and key enzyme of pentose phosphate metabolic pathway and it is widespread in all tissues and blood cells, catalyzing the conversion of glucose 6-phosphate to 6-phosphoglucono-δ-lactone in the presence of $NADP^+$ (Lehninger, 2000). This reaction yields NADPH and D-ribose 5-phosphate. NADPH protects the cell against the oxidant agents by producing reduced glutathione (Lehninger, 2000; Bianchi *et al.*, 2001). NADPH is also a coenzyme participating in the synthesis of a number of biomolecules such as fatty acids, steroids, and some amino acids (Kahler and Kirkman, 1983; Takizawa *et al.*, 1986). D-ribose 5-phosphate and its derivatives are components of DNA, ATP, CoA, NAD^+, FAD, and RNA (Keha and Kufrevioglu, 2000). In the case of NADPH lack, the concentration of reduced glutathione in a living system declines, resulting in cell death. For this reason, G6PD can be defined as an antioxidant enzyme (Srivastava and Beutler, 1989; Kozar *et al.*, 2000).

Many drugs are known to activate or inhibit several body enzymes (Edward and Morse, 1988; Jacobasch and Rapoport, 1996; Beydemir *et al.*, 2000) affecting the metabolic pathways. If any drug inhibits G6PD, the decreased NADPH, D-ribose 5-phosphate and GSH will cause cell damage in cells, resulting in severe health problems (Beutler, 1971; Jacobasch and Rapoport, 1996).

No studies can be found on the *in vitro* and *in vivo* effects of penicillin, sulbactam, cefazolin, and amikacine on the sheep liver G6PD activity.

This study was aimed to determine any possible effect of some commonly used antibiotics on the sheep liver G6PD activity.

MATERIAL AND METHODS

Material

2',5'-ADP Sepharose 4B was purchased from Pharmacia. $NADP^+$, glucose 6-phosphate, protein assay reagent were purchased from Sigma Chem.

Co. All other chemicals used were analytical grade and purchased from either Sigma or Merck.

Preparation of the homogenate

35 g of fresh sheep liver was first cut into small pieces. Excess blood sample was removed by ice-cold saline, and membranes were removed. The 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 the top speed for 3 minutes. Then the material was homogenized using an ultrasonic homogenizer for 40 minutes. Afterward, the homogenate was centrifuged at 12 100 rpm for 60 min, and the precipitate was removed. This process was repeated three times and a temperature of +4°C was maintained during the process (Mohammed *et al.*, 1976).

Preparation of affinity gel

Two grams of dried 2',5'-ADP Sepharose 4B gel were used for 10 ml column volume. The gel was washed with 400 mL distilled water to remove foreign bodies and the air of swollen gel was eliminated. The gel was suspended in 0.1 M K-acetate/ 0.1 M K-phosphate buffer (pH 6.0); then it was packed in a small column (1 × 10 cm) and equilibrated with the same buffer. The gel was washed with equilibration buffer. The flow rates for washing and equilibration were adjusted with peristaltic pump to 50 ml/h (Ninfali *et al.*, 1990).

Purification of G6PD by affinity chromatography

The homogenate sample obtained previously was loaded on 2', 5'-ADP Sepharose 4B affinity column and the flow rate was adjusted to 20 ml/h. Then the column was sequentially washed with 25 ml of 0.1 M K-acetate + 0.1 M K-phosphate, (pH 6.0) and 25 ml 0.1 M K-acetate + 0.1 M K-phosphate (pH 7.85). The washing with 0.1 M KCl + 0.1 M K-phosphate (pH 7.85) was continued until the final absorbance difference was 0.05. Elution was carried out with 80 mM K-phosphate + 80 mM KCl + 0.5 mM $NADP^+$ + 10 mM EDTA (pH 7.85). The enzyme activity was measured in final fractions, and the activity-containing tubes were collected together. Then the enzyme solution was dialyzed in 50 mM K-acetate plus 50 mM K-phosphate buffer (pH 7.0) for 2 h with two exchanges of buffer (Muto and Tan, 1985; Ninfali *et al.*, 1990). All of the procedures were performed at 4°C.

SDS polyacrylamide gel electrophoresis (SDS-PAGE)

The control of enzyme purity, using Laemmli's procedure (Laemmli, 1970), was carried out in 4% and 10% acrylamide concentrations for stacking and running gel, respectively. 10% SDS was added to the gel solution. The gel was stabilized in a solution containing 50% propanol + 10% TCA + 40% distilled water for 30 minutes. Staining was made for about 2 h in a solution of 0.1% Coommassie Brillant Blue R-250 + 50% methanol + 10% acetic acid. Finally, washing was carried out in a solution of 50% methanol + 10% acetic acid + 40% distilled water until protein bands were cleared.

Activity determination

The enzymatic activity was measured by Beutler's method (Beutler, 1971). One enzyme unit was defined as the enzyme amount reducing 1μmol $NADP^+$ per 1 min at 25°C and optimal pH (pH 8.0).

Protein determination

Quantitative protein determination was measured spectrophotometrically at 595 nm according to Bradford's method (Bradford, 1976), with bovine serum albumin as a standard.

In vitro drug effect

In order to determine the effects of some drugs on G6PD, some concentrations of penicillin (8.06, 16.12, 24.18, 32.24, and 40.30 mM), sulbactam (7.69, 15.38, 23.07, 30.76, and 38.45 mM), cefazolin (5.50, 13.75, 27.50, 41.25, and 55.00 mM) and amikacine (21.35, 32.02, 47.70, 53.37, and 64.05 mM) were added to separate tubes containing 50 μl purified enzyme, 0.2 mM $NADP^+$,

0.6 mM G6-P, 0.1 M Tris-HCl (pH 8.0), 10 mM $MgCI_2$ in a total volume of 1 ml. The enzyme activity was measured in these tubes, taking the tubes using no drug as control (100% activity). The I_{50} values were obtained after activity in % was plotted vs. drug concentration.

Statistical analysis

Data were presented as means ± standard deviation (S.D.). Three parallel measurements were analyzed by Student's *t*-test. Kruskal-Wallis One Way Analysis of Variance was used for comparisons of means. *P* values < 0.05 were accepted as statistically significant. Drug concentrations that produce 50% inhibition (I_{50}) were calculated from [Drug]-activity % graphs.

RESULTS AND DISCUSSION

Glucose 6-phosphate dehydrogenase catalyzes the conversion of glucose 6-phosphate to 6-phosphogluconate in the presence of $NADP^+$. GSH is used by antioxidant defence mechanisms and produced by NADPH synthesized in the pentose phosphate metabolic pathway in which G6PD and 6PGD participate (Beutler, 1994; Lehninger, 2000). For this reason, one can consider G6PD as an antioxidant enzyme. Therefore, this enzyme is very important for living cells.

A lot of drugs have adverse effects on the organism and its enzymes when used for therapeutic or other purposes (Hochster *et al.*, 1972). The effects can be dramatic and systemic (Christensen *et al.*, 1982). Similarly, acetazolamide inhibits carbonic anhydrase (CA), giving rise to severe diuresis (Warnock, 1989). It is reported that human red blood cell G6PD is affected by sodium ampicillin, netilmycin sulphate (Çiftçi *et al.*, 2000) and metamizol (Çiftçi *et al.*, 2001), and human red blood cell CA by sodium ampicillin and metamizol (Beydemir *et al.*, 2000). Moreover, the effects of halothane on G6PD of mouse liver and red cells were investigated and it is reported that both enzyme activities are decreased by halothane similarly like *in vivo* (Belge *et al.*, 2000). A lot of antibiotics are commonly used for animal disease therapy but their effects on G6PD enzyme activ-

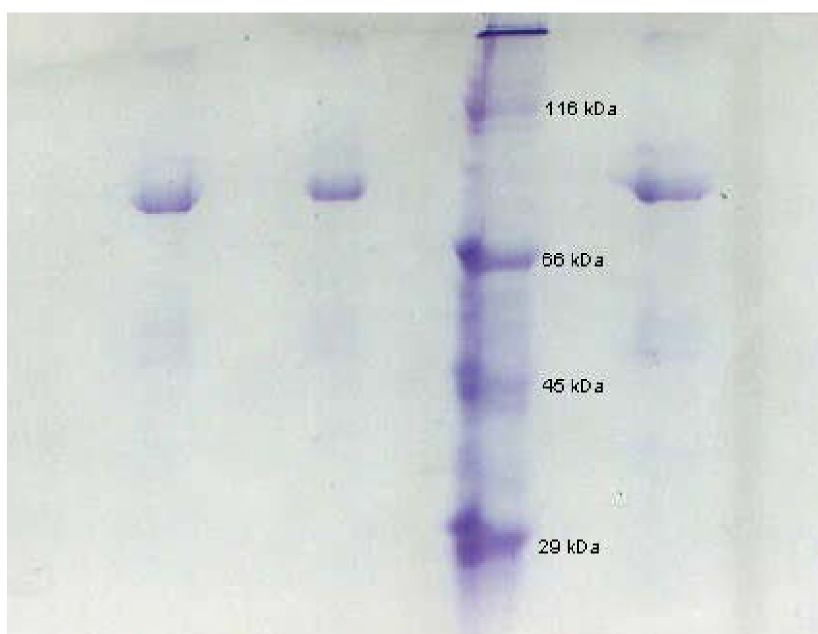

Figure 1. SDS-polyacrylamide gel electrophoresis of G6PD purified by affinity gel. Lane 1, 2 and 4: sheep liver G6PD (84 000 Da); Lane 3: standard proteins (Standards: *E. coli* β-galactosidase (116 000 Da), bovine albumin (66 000 Da), chicken ovalbumin (45 000 Da), and bovine carbonic anhydrase (29 000 Da)

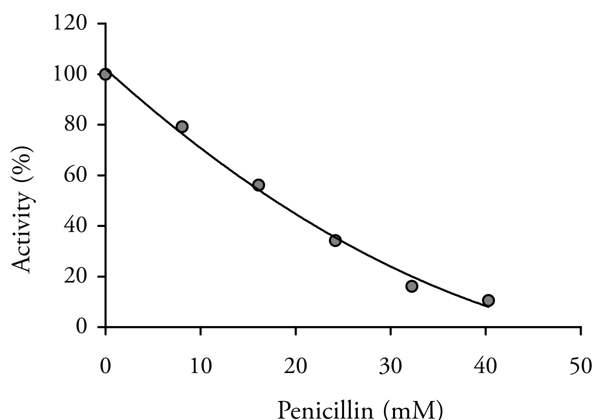

Figure 2. Activity % vs. penicillin concentration regression analysis graphs for G6PD in the presence of 5 different penicillin concentrations

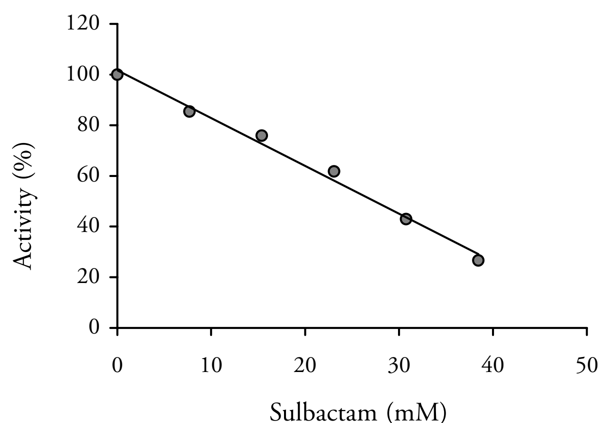

Figure 3. Activity % vs. sulbactam concentration regression analysis graphs for G6PD in the presence of 5 different sulbactam concentrations

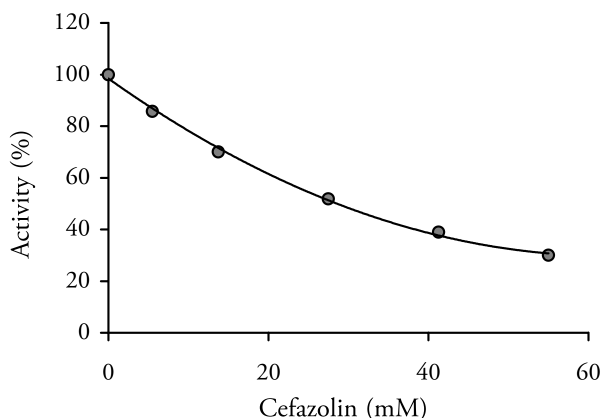

Figure 4. Activity % vs. cefazolin concentration regression analysis graphs for G6PD in the presence of 5 different cefazolin concentrations

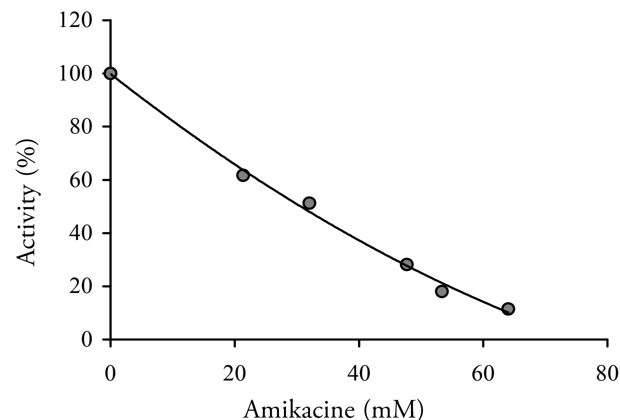

Figure 5. Activity % vs. amikacine concentration regression analysis graphs for G6PD in the presence of 5 different amikacine concentrations

ity are not known. In the present study, the effects of some commonly used antibiotics on sheep liver G6PD were investigated *in vitro*.

For this purpose sheep liver G6PD was purified 1 913 times with a yield of 35.72% by using 2',5'-ADP Sepharose 4B affinity gel chromatography. To control the purification of the enzyme, SDS-PAGE was performed. As shown in Figure 1, the enzyme (Lane 1, 2 and 4) has a single band after the affinity chromatography step.

Penicillin, sulbactam, cefazolin and amikacine inhibited the G6PD enzyme activity *in vitro*. The statistically significant differences ($P < 0.005$) in the mean values between the treatment groups were estimated by the analysis of variance. I_{50} value was determined from [Drug]-activity % graphs (Figures 2–5).

In order to show inhibitory effects, I_{50} value is a suitable parameter, and a lot of researchers use it. Therefore, I_{50} parameters of these antibiotics for G6PD were determined in this study.

Amikacine is an aminoglycoside antibiotic, commonly used for disease therapy. However, it has some side effects such as nephrotoxicity, ototoxicity, neurotoxicity, fever, bone marrow depression,

and hemolytic anemia. Ampicillin and cefazolin are beta-lactam antibiotics, having many side effects (fever, allergy, epidermal eruption). Sulbactam is a cephalosporin antibiotic and its side effects include fever, allergy, epidermal eruption (Kayaalp, 1998).

It is evident from Figures 2–5 that the selected antibiotics showed different inhibitory effects on the G6PD enzyme activity in sheep liver. The I_{50} values of penicillin, sulbactam, cefazolin and amikacine are 17.71, 27.38, 28.88, and 30.59 mM, respectively. According to the results, antibiotics used in the experiments are potent inhibitors for sheep liver G6PD. The I_{50} value for ampicillin is lower than those for the other antibiotics, suggesting the higher inhibitory effect of ampicillin. Declining effects were demonstrated for sulbactam, cefazolin and amikacine, respectively.

In the case of application of penicillin, sulbactam, cefazolin and amikacine to the patient, their dosage should be very well ordered to decrease the hemolytic side effects.

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