The effects of some antibiotics on sheep lens glucose 6-phosphate dehydrogenase *in vitro*

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PURPOSE. *To investigate the* in vitro *effects of gentamicin sulfate, vancomycin hydrochloride, sodium cefazolin and ceftriaxone on glucose 6-phosphate dehydrogenase enzyme (G6PD) purified from sheep lenses.*

METHODS. *G6PD was purified from sheep lenses with a yield of 66.8% and a specific activity of 7.8 U/mg proteins, and 10,400-fold using ammonium sulfate fractionation and 2′,5′-ADP Sepharose 4B affinity gel. The enzyme activity was determined by Beutler's method.*

RESULTS. *Gentamicin sulfate and vancomycin hydrochloride strongly inhibited the enzyme* in vitro*. The concentrations causing 50% inhibition (IC_{50}) were 15.34, and 8.0 mM, respectively. Conversely, cefazolin sodium strongly activated this enzyme, and ceftriaxone caused milder activation.*

CONCLUSIONS. *If a patient with G6PD deficiency requires gentamicin sulfate or vancomycin hydrochloride, routine ophthalmic did not inhibit this enzyme. Postmortem studies are now needed to investigate the activity of G6PD and how it is affected by these antibiotics. (Eur J Ophthalmol 2003; 13: 155-61)*

KEY WORDS. *Sheep lens, Antibiotics, Cataract, Glucose-6-phosphate dehydrogenase deficiency*

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INTRODUCTION

Glucose-6-phosphate dehydrogenase (D-glucose 6-phosphate: $NADP^+$ oxidoreductase, EC 1.1.1.49; G6PD) catalyzes the first and rate-limiting reaction of the pentose phosphate metabolic pathway which is a unique source for NADPH synthesis with concomitant reduction of $NADP^+$ in cells (1). This enzyme is present in all mammalian tissues and is found only in the cytoplasmic compartment (2). Data on G6PD activity are available for many tissues of humans, other mammals, plants and microorganisms (3-5). The NADPH generated by the pentose phosphate metabolic pathway is essential for many biosynthesis reactions, for the protection of cells against oxidative damage, and for pentose phosphates for nucleotide synthesis (6-8).

Many enzymes are important for maintaining the integrity of the lens metabolism in the eye (9). The pentose phosphate metabolic pathway functions in the lens to generate reduced equivalents (NADPH), used mainly for the maintenance of reduced glutathione (GSH) levels (10-12). Lens G6PD activity has been investigated in mammals such as bovines and rats (13-15). G6PD deficiency may arise in all tissues, including the crystalline lens (16). This deficiency may be due to a genetic abnormality, age or diet (17, 18).

When the ratio rises, cataract risk increases with G6PD deficiency in the lens. A number of studies have investigated the relationship between cataractogenesis and G6PD deficiency. For instance, Orzalesi et al. reported G6PD deficiency was a predisposing factor for the development of cataract (19). Etiologic factors such as heredity, latitude and exposure to light are also important in cataract development (16).

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Many antibiotics are used to deal with eye disorders but there are few studies of their effects on enzyme activities. Some studies found either increases or decreases in mammalian enzyme activities and the inhibitory effects of s antibiotics such as odium ceftizoxime, sodium ampicillin, sodium cefuroxime, sodium cefazolin, sodium cefoperazone, streptomycin sulfate, gentamicin sulfate, and netilmicin sulfate have been investigated in human erythrocytes *in vitro* and rat erythrocytes *in vivo* on G6PD enzyme activity (20). To our knowledge the effects of some widely used antibiotics on lens G6PD have not been investigated. The present study therefore investigated in vitro the effects of gentamicin sulfate, vancomycin hydrochloride, sodium cefazolin and ceftriaxone on G6PD purified from sheep lenses.

Gentamicin sulfate is popular among ophthalmologists, and is widely used. Its bactericidal activity is achieved by binding bacterial RNA polymerase and blocking protein synthesis. It is effective against most Gram-negative bacteria, including *Pseudomonas aeruginosa*. It is active against *staphylococci* and some *streptococci* but not against *pneumococci* (21). Gentamicin sulfate can be given topically, subconjunctivally and systemically (22).

Vancomycin hydrochloride is a bactericidal antibiotic which is highly effective against staphylococci that have become resistant to all other drugs. Its spectrum includes nearly all Gram-positive organisms (23). The use of vancomycin hydrochloride, if given intravenously, is limited by nephrotoxicity and ototoxicity, and subconjunctival injections can cause sloughing. However, topical use and intravitreal use appear to be well tolerated (24).

The cephalosporins are bactericidal and exert their effect by interfering with cell wall synthesis. A large number of variations of the molecule are available commercially and, depending on when they were developed, are classified by generations. First-generation cephalosporins (eg, sodium cefazolin) are only moderately effective against some Gram-negative enterobacilli, but are highly effective against *staphylococci* and other Gram-positive cocci. Some cephalosporins of the third generation are particularly effective against *Pseudomonas* (eg, ceftriaxone). None of the cephalosporins are very effective against methicillin-resistant *staphylococci* or *enterococcal streptococci* (25).

We investigated G6PD activity after exposure to some antibiotics that are commonly used in the treatment of eye infections, to identify any adverse effects such as inhibition of G6PD which is believed to be related to cataract formation.

MATERIALS AND METHODS

Materials

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

In vitro studies

Preparation of the homogenate

The lenses were extracted in cool conditions from sheep lenses collected from a local slaughterhouse. The lenses were washed three times with 10 mM Tris-HCl buffer (pH 7.6) containing 1 mM 2-mercaptoethanol (2-ME), homogenised in liquid nitrogen and mixed with 10 mM Tris-HCl buffer, as above, then centrifuged at 4°C, 39100 x g for 30 min. The supernatant was used in further studies (26).

Ammonium sulphate fractionation and dialysis

The hemolysate was precipitated with ammonium sulphate (0-10%, 10-20%, 20-30%, 30-40%, 40-50%, and 50-60%) and at each step, G6PD activity was determined in the supernatant and the precipitate. The enzyme precipitated at 0-30% ammonium sulphate. The enzyme solution was dissolved in a small amount of 50 mM phosphate buffer (pH 7.0), and dialyzed at 4°C in 50 mM K-acetate/50 mM K-phosphate buffer (pH 7.0) for 2 h with two changes of buffer (27).

Purification of G6PD by affinity chromatography

Two grams of dried 2′,5′ADP-Sepharose 4B gel were resuspended in 0.1 M K-acetate/0.1 M K-phosphate

buffer (pH 6.0), and used to pack a small column (1 x 10 cm) which was equilibrated in the same buffer. The dialysed sample was loaded onto a 2′,5′ADP Sepharose 4B affinity column and the gel was washed with 25 mL of 0.1 M K-acetate/0.1 M K-phosphate (pH 6.0), with 25 mL of the same buffer at pH 7.85, and finally, with 0.1 M KCl/0.1 M K-phosphate (pH 7.85). Washing was continued until absorbance was 0.05 at 280 nm. Elution was carried out with 80 mM K-phosphate + 80 mM KCl + 0.5 mM $NADP^+$ + 10 mM EDTA (pH 7.85) solution at 20 mL/h flow rate. Elutes were collected in 2-mL tubes and the activity of each was calculated separately. Active fractions were collected. All procedures were done at 4°C (27, 28).

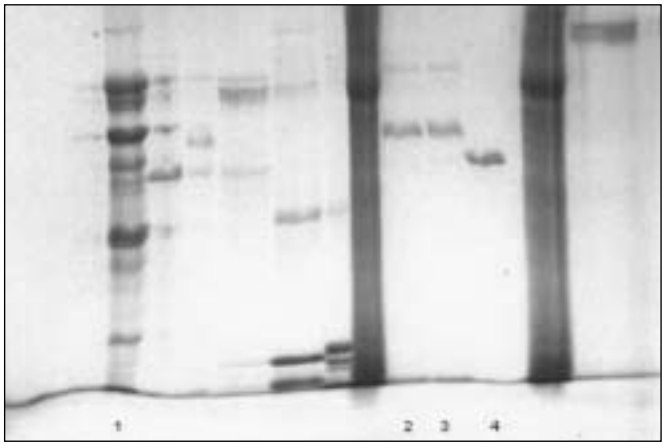

Fig. 1 - *SDS-polyacrylamide gel electrophoresis of G6PD purified by affinity gel. Lane 1 is the standard protein; lane 2 and 3 are sheep erythrocyte G6PD, lane 4 is sheep lens G6PD.*

Proteins

Protein was quantitatively determined by spectrophotometry at 595 nm according to Bradford's method, with bovine serum albumin as standard (29).

SDS polyacrylamide gel electrophoresis

SDS polyacrylamide gel electrophoresis was done after purification of the enzyme according to Laemmli's method (30). It was carried out in 4% and 10% acrylamide respectively for stacking and running gels, containing 0.1% SDS. As samples, standard proteins, sheep erythrocyte G6PD, and sheep lens G6PD were applied to the electrophoresis medium. Gel was stained overnight in 0.1% Coomassie Brilliant Blue R-250 in 50% methanol and 10% acetic acid, then destained by frequently changing the same solvent, without dye. The electrophoretic pattern was photographed (Fig. 1).

Effects of antibiotics

The *in vitro* effects of drugs on the activity of G6PD were investigated as follows: 580 µL of distilled water, 20 µL of sheep lens G6PD pure enzyme sample, 100 µL of 2 mM $NADP^+$, 100 µL of 0.1 M $MgCl_2$, and 100 µL of 1 M Tris-HCl buffer (pH 8.0) containing 5 mM EDTA were added in the blind cuvette, and 100 µL of 6 mM glucose 6-phosphate (G6P), sheep lens G6PD pure enzyme sample, $NADP^+$, $MgCl_2$, and Tris-HCl buffer containing 5 mM EDTA in the same amounts as above were added in the sample cuvette. The activity was measured with a spectrophotometer at 340 nm, at five different cuvette concentrations. Drugless cuvette activity was taken as 100%. For each drug activity %-[drug] a graph was plotted at five different inhibitor concentrations, and the drug concentrations causing 50% inhibition (IC_{50}) were calculated The results are presented in Table I and Figures 2-5.

RESULTS

The overall purification of sheep G6PD gave a yield of 66.8%, with specific activity of 7.8 U/mg proteins, and increased 10,400-fold after ammonium sulfate fractionation and 2′,5′-ADP sepharose 4B affinity gel (Tab. II). SDS-PAGE was done after purification of the enzyme and the electrophoretic pattern was photographed (Fig. 1).

Both gentamicin sulfate and vancomycin hydrochloride *in vitro* strongly inhibited the G6PD activity of sheep lens. The IC_{50} for these two drugs are presented in Table I. Sodium cefazolin markedly activated this enzyme (Figure 4), and ceftriaxone stimulated G6PD activity mildly up to 1.37 mM compared with the control.

TABLE I - IC_{50} VALUES *IN VITRO*

Drug	IC^{50} (mM)
Gentamicin sulfate	15.34
Vancomycin HCl	8.0

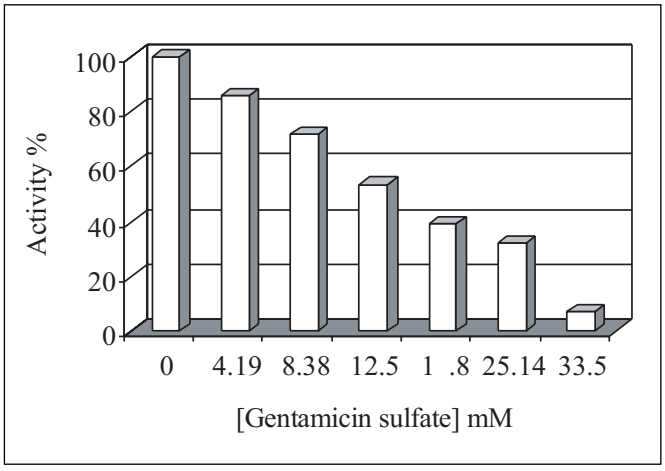

Fig. 2 - *Activity % - [drug] graph for G6PD in presence of gentamicin sulfate.*

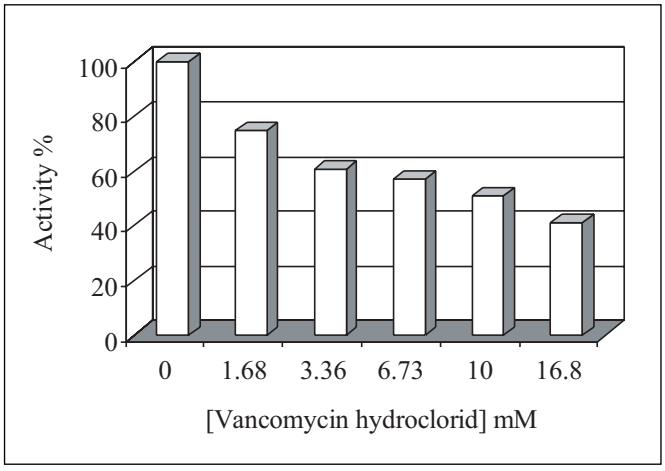

Fig. 3 - *Activity % - [drug] graph for G6PD in presence of vancomycin hydrochloride.*

DISCUSSION

Many chemicals at relatively low doses affect metabolism by altering normal enzyme activity, particularly through inhibition of a specific enzyme (31). The effects can be dramatic and systemic (32). For example, metamizol and magnesium sulfate have inhibitory effects on G6PD activity (33). Beydemir et al. reported the effects of some antibiotics on the activity of human erythrocyte carbonic anhydrase *in vitro* and rat erythrocyte carbonic anhydrase in vivo (34). In another study, halothane was found to inhibit red blood cell and liver G6PD activities significantly in the first week and still activated this enzyme in the second week after anesthesia compared with the control group (35).

G6PD deficiency is a widespread condition affecting more than 100 million people in the world (36). The life-span of people with G6PD deficiency is short on account of complications caused by chronic hemolysis. G6PD has approximately known 400 variants. As indicated in previous studies, the enzyme deficiency has been shown in tissues including the lens, especially in subjects with the Mediterranean type of deficiency (37-40). Heredity and red blood cell (RBC) abnormalities may cause G6PD deficiency, possibly in view of the cytologic and metabolic similarities between RBC and the cells of the lens (36). The pentose phosphate metabolic pathway is affected by the G6PD deficiency, shortening the ribose available for the synthesis of nucleic acids and reducing the renewal of lens proteins (41).

In cases with enzyme deficiencies therapeutic agents must be chosen carefully because in infectious diseases accompanied by G6PD deficiency, antibiotics such as sulphamethoxasole may cause hemolytic anemia of RBCs (42). G6PD seems to be needed in the defense mechanism against free radicals generated in the metabolism of some drugs.

On account of the cytologic and metabolic similarities between RBC and the cells of the lens some antibiotics inhibit G6PD and cause cataract. Orzalesi et al reported G6PD deficiency was a predisposing factor for the development of cataract (19). Yüreğir et al found 52% of lens G6PD deficiency in a total of 52 patients with cataract (43). I Oxidative stress occurs in RBC and other tissues after treatment with some drugs in (44-46).

In our study, sheep lens G6PD was purified 10,400-fold by using ammonium sulfate fractionation and 2′,5′-ADP Sepharose 4B affinity gel. SDS-PAGE was appropriate for the purity and molecular weight of the enzyme (Fig. 1) and a high-purity enzyme was obtained. As seen in the gel photograph, sheep erythrocyte G6PD and sheep lens G6PD have different molecular weights (66880 and 54957 Dalton).

In ophthalmology drugs can be administered by several routes. Topical, peribulbar or intraocular administration may not be effective for all eye diseases. Systemic medication is often required to achieve effec-

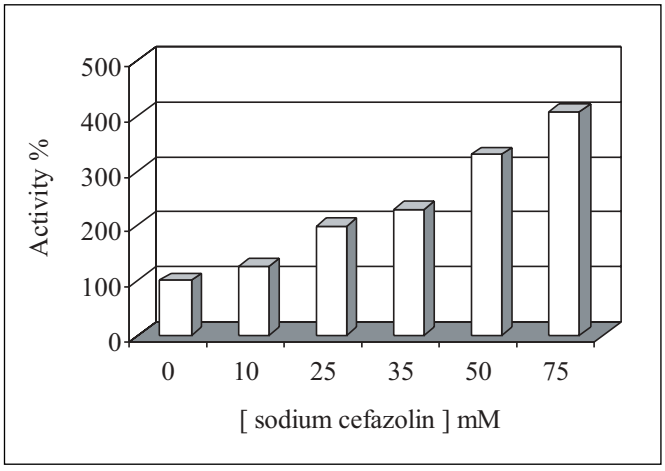

Fig. 4 - *Activity % - [drug] graph for G6PD in presence of sodium cefazolin.*

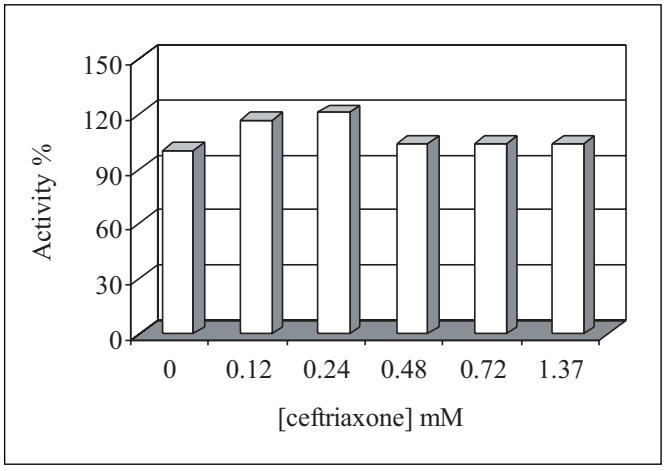

Fig. 5 - *Activity % - [drug] graph for G6PD in presence of ceftriaxone.*

tive concentrations of a drug in and around the ocular tissues. Systemic antimicrobial agents are used for anterior, posterior segment or orbital infections, often in conjunction with topical treatment. Some conditions such as acute dacryocystitis, gonococcal and chlamydial conjunctivitis, Pseudomonas keratitis, lid trauma, preseptal cellulitis, orbital cellulitis, endophthalmitis and penetrating eye injury prophylaxis require concurrent systemic treatment.

Gentamicin sulfate has proven effects in the treatment of P. aeruginosa keratitis (47). In this study we found that gentamicin sulfate inhibited G6PD activity. It may play a role in cataractogenesis. Ceftriaxone, however did not affect the enzyme. In the last few years, Pseudomonas has shown increasing resistance (48) so it may be useful to replace gentamicin with ceftriaxone for the treatment of intraocular infections.

Vancomycin hydrochloride should not be used indiscriminately, as we may be left without a defense against multiresistant *staphylococci* if resistance develops (49). Because of the resistance problem (49) and the lesser problem of enzyme inhibition suggested in our study, vancomycin hydrochloride may be a second choice after ceftriaxone in ocular infections, especially for patients with G6PD deficiency.

The effects of some commonly used antibiotics on lens G6PD were investigated *in vitro*. Ceftriaxone caused mild activation of the enzyme, while sodium cefazolin

TABLE II - PURIFICATION OF GLUCOSE 6-PHOSPHATE DEHYDROGENASE FROM SHEEP LENS

Purification step	Activity (EU/ml)	Total volume (ml)	Protein (mg/ml)	Total protein (mg)	Total activity (EU)	Specific activity (EU/mg)	Yield (%)	Purification factor
Homogenate	0.0117	57	15.6	577.2	0.6669	0.00075	100	1
Ammonium sulfate precipitation (0-30 %)	0.118	5	7.16	21.48	0.590	0.0164	88.46	22
2´,5´- ADP Sepharose 4B affinity chromatography	0.193	3	0.0248	0.0744	0.579	7.8	86.82	10,400

activated it strongly. The study showed that vancomycin hydrochloride and gentamicin sulfate have strong inhibitory effects on lens G6PD, the inhibitory effect of gentamicin sulfate (IC_{50} 15.34 mM) being entirely consistent with a previous study by Çiftçi et al (20). They found that gentamicin sulfate strongly inhibited this enzyme in human erythrocytes (IC_{50} 12 mM, K_i 4.66 x 10^{-3} M). However, sodium cefazolin also has an inhibitory effect on human erythrocyte G6PD (IC_{50} 125, K_i 1.19 x 10^{-1} M) (20). However, in our study, sodium cefazolin activated this enzyme. There may be different levels of activity of erythrocyte and lens enzymes and the effects against some inhibitors may vary as well.

Vancomycin hydrochloride remains an extremely effective last-resort drug for serious ocular infections like keratitis, endophthalmitis or orbital cellulitis. However, since there may soon be no remaining effective antibiotics, it must be used very carefully whereas sodium cefazolin and ceftriaxone may be used safely as they showed no inhibitory effect. If a patient with G6PD deficiency requires gentamicin sulfate and vancomycin hydrochloride, routine ophthalmic examinations should be done.

Postmortem studies are now needed to examine the enzyme activity in human lenses.

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