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Research Article

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The Role Of Antibiotics in The Management of the Polyol Pathway: an *in Vitro* and *in Silico* Approach

Poliol Yolunun Kontrolünde Antibiyotiklerin Rolü: Bir *in Vitro* ve *in Siliko* Yaklaşım

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ABSTRACT

Increased activity of aldose reductase (AR) and sorbitol dehydrogenase (SDH) are the major causes of diabetic complications. Thus, inhibition of these two enzymes is vital in preventing diabetic complications. As the synthesis of new and effective AR and SDH enzyme inhibitors is quite difficult, we have investigated the inhibition effects of antibiotics, which are already widely used in medicine, on AR and SDH enzymes. AR and SDH enzymes were purified from bovine kidney, in vitro effects of antibiotics on enzymes were determined, and molecular docking simulations were carried out to understand inhibition mechanisms. The antibiotics ampicillin and amikacin inhibited both AR and SDH enzymes at very low concentrations. The best inhibitors for AR were found to be ceftriaxone, tylosin, and metronidazole with IC_{50} values of 28.75 µM, 49.28 µM and 58.42 µM, respectively. The best inhibitors for SDH were seen to be amikacin, ampicillin, and ceftazidime with IC_{50} values of 2.4 mM, 2.62 mM, and 3.76 mM, respectively. The results of inhibition and docking studies showed that antibiotics are highly effective on these enzymes. The results obtained can be used as a reference for synthesizing better inhibitors in future studies.

Key Words

Aldose reductase; molecular docking; polyol pathway; sorbitol dehydrogenase.

ÖZ

Aldoz redüktaz (AR) ve sorbitol dehidrogenazın (SDH) artan aktivitesi, diyabetik komplikasyonların başlıca nedenleridir. Bu nedenle, bu iki enzimin inhibisyonu, diyabetik komplikasyonların önlenmesinde hayati önem taşımaktadır. Çalışmamızda, yeni ve etkili AR ve SDH enzim inhibitörlerinin sentezi oldukça zor olduğundan, halihazırda tıpta yaygın olarak kullanılan antibiyotiklerin AR ve SDH enzimleri üzerindeki inhibisyon etkileri araştırılmıştır. AR ve SDH enzimleri sığır böbreğinden saflaştırılmış, antibiyotiklerin enzimler üzerindeki in vitro etkileri belirlenmiş ve inhibisyon mekanizmalarının aydınlatılması amacıyla moleküler docking simülasyonları gerçekleştirilmiştir. Ampisilin ve amikasin antibiyotikleri hem AR hem de SDH enzimlerini çok düşük konsantrasyonlarda inhibe etmiştir. AR için en iyi inhibitörlerin sırasıyla 28.75 µM, 49.28 µM ve 58.42 µM IC_{50} değerleri ile seftriakson, tylosin ve metronidazol antibiyotikleri olduğu bulunmuştur. En iyi SDH inhibitörlerinin sırasıyla 2,4 mM, 2,62 mM ve 3,76 mM IC_{50} değerleri ile amikasin, ampisilin ve seftazidim olduğu görülmüştür. İnhibisyon ve docking çalışmalarının sonuçları, antibiyotiklerin bu enzimler üzerinde oldukça etkili olduğunu göstermiştir. Elde edilen sonuçlar, gelecekteki çalışmalarda daha iyi inhibitörlerin sentezlenmesi için referans olarak kullanılabileceği düşünülmektedir.

Anahtar Kelimeler

Aldoz redüktaz; moleküler docking; poliol yolu; sorbitol dehidrogenaz.

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INTRODUCTION

Diabetes Mellitus (DM) is a metabolic disease characterized by chronic hyperglycemia resulting in a high mortality and morbidity risk due to disruption in insulin secretion in β-cells of the pancreas and/or because of the disruption in the effect of insulin. The main reason for diabetes is the lack of circulating insulin or the reduction in the ability of the peripheral tissues to respond to insulin (insulin resistance). Chronic hyperglycemia in diabetes causes damage to many organs that include the eyes, nerves, heart, kidneys, and blood vessels [1, 2].

Under normal glycemic conditions, almost all of the glucose in a cell is phosphorylated to glucose 6-phosphate by the hexokinase enzyme. A very small amount of the non-phosphorylated part heads to the polyol pathway, which is an alternative pathway of glucose metabolism (Figure 1). Aldose reductase (AR, EC 1.1.1.21) is the first and rate-limiting enzyme of the polyol pathway, which converts glucose into sorbitol in the presence of the NADPH cofactor. Then, sorbitol is converted into fructose by the sorbitol dehydrogenase (SDH, EC 1.1.1.14) enzyme, which is the second enzyme of the polyol pathway [3].

The glucose that is reduced by AR causes sorbitol accumulation in some tissues. The conversion of glucose into fructose (the polyol pathway) not only increases the NADPH but also enhances the use of NAD^+. The polyol pathway activity, which is increased due to hyperglycemia, may lead to a decrease in the NADPH and reduced NAD^+ accumulation. The polyol-pathway changes in the pyridine nucleotides are connected to metabolic changes, such as nitric oxide (NO) synthesis and protein kinase activation [4].

Sorbitol accumulation causes osmotic stress increases, and the change in the NADPH/$NADP^+$ rate causes oxidative stress [5]. Another enzyme using NADPH is the nitric oxide synthase (NOS) enzyme. In hyperglycemia, the increase in the consumption of the NADPH in cells due to polyol pathway activation affects the production of GSH as well as NO synthesis. The NADPH amount decreasing due to the increase in the activity of AR enzymes causes a decrease in the NOS activity that catalyzes the NO formation in the arginine by using NADPH and reduces the endothelial NO synthesis [6]. Depending on the increased oxidative stress, the decreasing NO synthesis leads to atherosclerosis [7].

Together with the polyol pathway, the AR enzyme is associated with many other disorders, including cardiovascular diseases [8, 9], inflammation [10], depression [11, 12], renal failure [13, 14], ovarian anomalies [15, 16] and cancer [17, 18]. These regulatory effects of the AR enzyme increase the importance of inhibition studies conducted on this enzyme even more. AR inhibitors are used in the treatment of many diseases such as colon cancer and rheumatoid arthritis [5, 10, 18]. The Aldose reductase enzyme converts aldehydes of reactive oxygen species (ROS) into inactive alcohols and glucose into sorbitol in the presence of the NADPH cofactor. The depletion of NADPH in the cells where aldose reductase activity is high escalates oxidative stress by increasing the GSH level [19].

It was reported that aldose reductase enzyme inhibitors aldose reductase inhibitors'ın kısaltması olan (ARIs) increase motor nerve conduction velocity and the blood flow in sciatic nerves [20], preventing lens proteins from being damaged by avoiding the oxidative stress caused by the polyol pathway and increasing the GSH level [21]. In a study that was conducted on rat liver tumor cells, it was shown that aldose reductase ensures the uncontrolled division in the cell by suppressing the functionality of carbonyl metabolites (3-DG, glyceraldehyde). For this reason, it is considered that the increase in the expression of aldose reductase in liver tumor formation may be related to the inability to destroy cancer cells [22]. However, the roles of ARIs are not

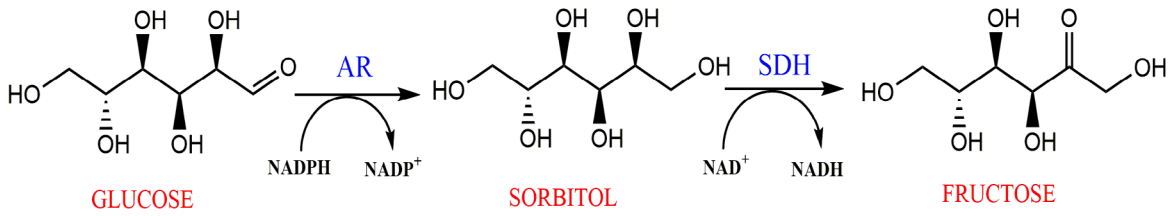

Figure 1. Polyol pathway which alternative way of glucose metabolism. The first enzyme of the pathway is aldose reductase and the second enzyme is sorbitol dehydrogenase. They use NADPH and NAD^+ as a coenzyme, respectively.

limited to these. In recent studies, it was shown that ARIs play roles as curative agents in the treatment of various cancers, including liver, breast, ovarian, cervix, and colon cancers [22, 17, 23, 13]. In addition, it was also reported that inhibition of sorbitol dehydrogenase, which is the second enzyme of the sorbitol pathway, helps prevent ischemia-reperfusion liver damage [24], and vascular hyperpermeability, which is one of the most important complications of retinopathy [25]. In addition, it was also shown that sorbitol dehydrogenase enzyme inhibitors delay the emergence of polyol-mediated diabetic complications with their ability to alleviate redox changes [26].

In light of all these data, it is apparent that aldose reductase, which is the first enzyme of the polyol pathway, and sorbitol dehydrogenase, which is the second enzyme of the polyol pathway, both play important roles in preventing or delaying diabetic complications. For this reason, determining the inhibitors of these enzymes is very important in terms of increasing the quality of life of diabetic patients and prolonging their life expectancy. Therefore, aldose reductase and sorbitol dehydrogenase enzymes were purified from the bovine kidney and the inhibition effects of frequently used antibiotics on these enzymes were examined in the present study. Also, to understand the inhibition mechanism of AR and SDH enzymes with antibiotics, molecular docking simulations were carried out.

MATERIALS and METHODS

Materials

Sephadex G-100, CM-Sephadex C-50, DEAE-Sephadex, β-nicotinamide adenine dinucleotide phosphate (β-NADPH), D-Sorbitol, protein assay reagents and chemicals for electrophoresis, DL-glyceraldehyde, β-$NADP^+$, and 2’5’-ADP Sepharose 4B were obtained from Sigma-Aldrich Co. (Taufkirchen, Germany). All other chemicals were analytical grade and obtained from Merck (Darmstadt, Germany). All drugs were provided from the University Hospital Pharmacy (Ataturk University, Erzurum, Turkey).

Preparation of the AR and SDH homogenates

The bovine kidney tissues obtained from a local slaughterhouse were finely chopped and washed with 0.9% NaCl to eliminate contaminants. Then, the kidney tissues were homogenized using a tissue homogenizer with a 10 mM Na-phosphate buffer containing 5 mM mercaptoethanol (pH 7.4). The homogenate samples obtained were centrifuged for 60 minutes at 13.500 g. Ammonium sulfate precipitation (0-70%) was carried out and centrifuged again for 60 minutes at 13.500 g. The precipitate was re-dissolved in a 10 mM Na-phosphate buffer (pH 7.4). Subsequently, the solution was dialyzed for three hours against a 10 mM Na-phosphate buffer (pH 7.4) containing 5 mM β-mercaptoethanol. The homogenate preparation process for the SDH enzyme was carried out in a similar way but, unlike the AR homogenate preparation process, a 5 mM K-phosphate buffer (pH=7.0) was used as a homogenate buffer.

Enzyme Activity Assays

The AR Activity Assay

The measurement of the AR enzyme activity was performed by modifying the procedure used by Cerelli et al. (1986) [27]. A 1 mL reaction medium was prepared by adding 0.1 mL of NADPH, 0.45 mL of deionized water, a Na-phosphate buffer of 0.25 mL, 0.1 mL of NADPH, a 0.1 mL isolated enzyme solution, and finally adding 0.1 mL of DL-glyceraldehyde to initiate the reaction. Enzyme activity was determined by spectrophotometrically monitoring the decrease in the NADPH concentration at 340 nm.

The SDH Activity Assay

SDH activity was measured by spectrophotometrically measuring the increase in absorbance resulting from the reduction of NAD^+ according to the D-sorbitol + NAD^+ ⇌ D-fructose + NADH + H^+ reaction. 10 mM of D-Sorbitol, 50 mM of glycine buffer (pH 9.9) and 470 mM β-NAD^+ were added to a quartz cuvette to prepare the reaction medium. The reaction was initiated by adding the enzyme solution to the reaction medium.

Purification of Enzymes

AR Purification

To purify the AR enzyme, a DE-52 cellulose ion-exchange chromatography, Sephadex G-100 gel filtration chromatography and 2’5’-ADP-Sepharose 4B affinity chromatography methods were conducted, respectively. The purification of the aldose reductase enzyme was conducted according to our previous study. The whole purification process was completed at 4°C [28]. The purity control of the enzyme was performed by SDS-polyacrylamide gel (SDS-PAGE) electrophoresis.

SDH Purification

The bovine kidney SDH enzyme was purified in three steps, including DEAE-Sephadex ion-exchange chromatography, CM-Sephadex C-50 ion-exchange chromatography, and Sephadex G-100 gel filtration chromatography. All chromatographic purification columns were prepared as previously reported by Alim and Beydemir [29]. The whole purification process was conducted at 4 °C. The purity control of the enzyme was performed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) [30].

Protein Determination

Following all purification steps, the protein amount was quantitatively determined according to the Bradford method. According to this method, protein samples give strong absorbance at 595 nm and bovine serum albumin is used as a standard protein [31].

Inhibition Studies

The inhibitory effects of the antibiotics cefazolin, ceftriaxone, cefuroxime, vancomycin, amikacin, gentamicin, cefotaxime, ampicillin, ceftazidime, chloramphenicol, metronidazole, lincomycin, ciprofloxacin, and tylosin on both AR and SDH enzymes were investigated. Five different concentrations of each antibiotic were prepared and the inhibition effects of these compounds were examined on both enzymes. The inhibitory-free control cuvette activity was considered to be 100% and inhibitor concentrations which are the half-maximal inhibitory concentration (IC_{50}) were determined. After the IC_{50} values were determined, K_i values were calculated for each inhibitor. To calculate the K_i values, enzyme activity values were determined for three different concentrations of the corresponding inhibitor against the 5 different substrate concentrations. These substrates are DL-glyceraldehyde for AR and D-sorbitol for SDH [32].

Molecular Docking Studies

Molecular docking simulations were carried out to determine the possible interactions of the studied drugs with target enzymes, AR and SDH. The Maestro 12.5 program, which is part of the Schrödinger Molecular Modeling Suite package program, was used to perform molecular docking simulations [33]. Firstly, X-ray crystal structures of AR (PDB ID: 2FZD) and SDH (PDB ID: 3QE3) enzymes were obtained from RCSB Protein Data Bank (PDB). Enzyme protein constructs were pre-processed and prepared using the Protein Preparation Wizard under physiological conditions. With protein preparation wizard [34], the binding order and charges were assigned, all missing hydrogen atoms were added**,** and using the Prime module of the Maestro 12.5, missing side chains of the protein were filled. 2D structure drawings of all ligands to be docked, their transformation into 3D structures, their protonation states at pH 7.4, and their optimizations were carried out with the help of the LigPrep program of Maestro. After preparing proteins and ligands to be docked, docking simulations of antibiotics against AR and SDH enzymes were performed using Glide/XP (extra precision) [35]. Binding energies and scores of the antibiotics to AR and SDH targets were calculated with the Glide program [36].

RESULTS and DISCUSSION

In our study, the bovine kidney AR enzyme was purified in four steps; purification was carried out by using ammonium sulfate precipitation (0-70%), DE-52 cellulose ion exchange, gel filtration, and 2',5'-ADP Sepharose 4B affinity chromatography methods. The AR enzyme was purified 9 times from the bovine kidney with these methods, with 0.444 EU/mg specific activity and with 1.78% yield (Table 1). In the present study, the bovine kidney SDH enzyme was purified in four stages. The purification was done by applying ammonium sulfate precipitation (40-70%), DEAE Sephadex ion exchange, CM-cellulose ion exchange, and gel filtration chromatography methods. With these methods, the bovine kidney SDH enzyme was purified approximately 170 times with 15.14 EU/mg specific activity and a 4.11% yield (Table 2).

The purity of the enzymes was checked by the SDS-PAGE method and is shown in figure 2. In addition, the molecular weights of the enzymes were calculated by plotting log MW-Rf graphs. The molecular weight of the AR and SDH enzymes were calculated as 37.50 kDa (Figure 2c), and 37.56 kDa (Figure 2d), respectively.

In the inhibition studies on the AR enzyme, the best inhibition effect was determined in the ceftriaxone compound that had 28.75 µM. Ceftriaxone was followed by tylosin and metronidazole compounds whose IC_{50} values were 49.28 µM and 58.42 µM, respectively. Lincomycin, gentamicin and cefazolin showed the lowest inhibition effect with IC_{50} values of 345 mM, 69 mM and 14.74 mM, respectively. In addition, K_i values

Table 1. Purification steps of aldose reductase from bovine kidney

Purification Steps	Activity (EU/ml)	Protein (mg/ml)	Total volume (ml)	Total activity (EU)	Total Protein (mg)	Specific activity (EU/mg)	Purification fold	Yield %
Homogenate	0.15	3.092	45	6.75	139.14	0.048	1	100
$(NH_4)_2SO_4$ precipitation and dialysis	0.18	3.24	30	5.40	97.2	0.055	1.14	80
DE-52 cellulose ion-exchange chromatography	0.128	1.435	20	2.56	28.7	0.089	1.85	37.9
Gel filtration chromatography	0.043	0.27	15	0.64	4.05	0.159	3.31	9,48
2'5'-ADP-Sepharose 4B affinity chromatography	0.012	0.027	10	0.12	0.27	0,444	9.15	1.78

Table 2. Purification steps of sorbitol dehydrogenase from bovine kidney

Purification Steps	Activity (EU/ml)	Protein (mg/ml)	Total volume (ml)	Total activity (EU)	Total Protein (mg)	Specific activity (EU/mg)	Purification fold	Yield %
Homogenate	0.177	1.992	45	7.96	89.64	0.089	1	100
$(NH_4)_2SO_4$ precipitation and dialysis	0.187	1.815	16	2.99	29.04	0.103	1.16	37.5
DEAE-Sephadex ion-exchange chromatography	0.166	0.021	15	2.49	0.321	7.750	87.27	31.3
CM-Cellulose ion-exchange chromatography	0.160	0.013	15	2.40	0.192	12.50	140.76	30.1
Gel filtration chromatography	0.041	0.004	8	0.33	0.0366	15.14	170.5	4.11

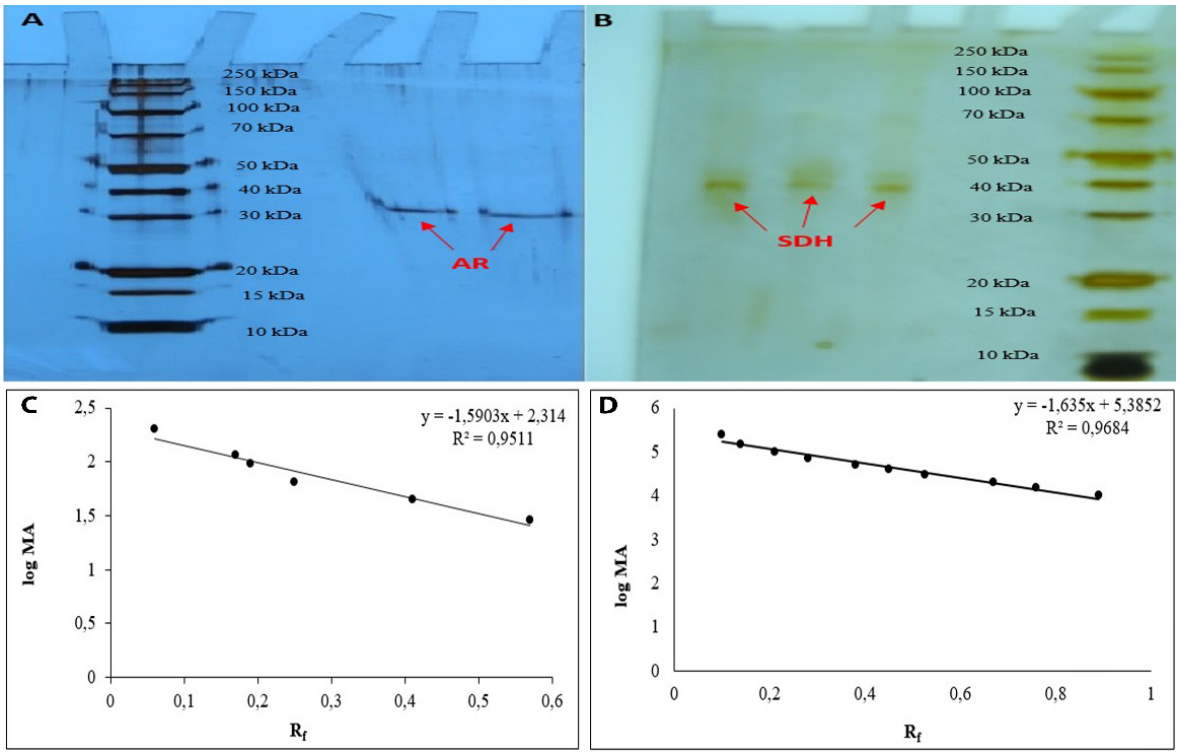

Figure 2. Purity control of AR and SDH enzymes by polyacrylamide gel electrophoresis (SDS-PAGE) method. The A and B columns show results for AR and SDH, respectively. log MW-Rf plots for calculating the molecular weights of the AR (C) and SDH (D) enzymes.

of cefazolin, ceftriaxone, cefuroxime, vancomycin, amikacin, gentamicin, cefotaxime, ampicillin, ceftazidime, and chloramphenicol antibiotics, which show inhibition effect on AR enzyme, were determined as 15.38 ± 4.86 mM, 52.61 ± 11 µM, 3.21 ± 0.3 mM, 0.106 ± 0.01 mM, 7.54 ± 1.3 mM, 52.82 ± 12.7 mM, 2 ± 0.2 mM, 2.72 ± 0.7 mM, 1.39 ± 0.1 mM, 3.07 ± 1.3 mM, respectively. The AR enzyme inhibition results are given as a whole in Table 3. In inhibition studies we conducted on the SDH enzyme, the amikacin compound showed the best inhibition effect with an IC_{50} value of 2.4 mM. Amikacin was followed by the ampicillin and ceftazidime compounds with IC_{50} values of 2.62 mM and 3.76 mM, respectively. The lowest inhibition effect was shown in lincomycin and gentamicin with IC_{50} values of 72 mM and 38.5 mM, respectively. In addition, Ki values of amikacin, gentamicin, ampicillin, and lincomycin antibiotics, which show inhibition effect on SDH enzyme, were determined as 2.41 ± 0.3 mM, 19.91±4.3 mM, 2.06 ±0.2 mM, 40.33 ±5.1 mM, respectively. The SDH enzyme inhibition results are given in Table 3.

In addition, molecular docking simulations were performed to examine the theoretical interactions of the antibiotics with AR and SDH enzymes and to understand their inhibition mechanisms. The molecular docking scores and binding energy results for AR and SDH enzymes are given in Table 4 and Table 5, respectively.

As it is already known, diabetes is a common metabolic syndrome and is characterized by hyperglycemia. This metabolic disease is associated with many degenerative complications including nephropathy [37], neuropathy [38], cataract [39], retinopathy [40], increased myocardial infarction and increased stroke risk because of atherosclerosis [41]. The probability of these pathologies is quite high in both Type I and Type II diabetes. The control and prevention of these complications for quality of life and mortality rates of diabetic patients is a very important and serious therapeutic problem [42, 43]. Studies have shown that aldose reductase and sorbitol dehydrogenase enzymes inhibitors play important roles in preventing or delaying diabetic complications [44, 45].

However, only a few of the aldose reductase inhibitors, which were included in clinical tests fit clinical profiles. Sorbinil, which is an aldose reductase inhibitor, with

Table 3. IC_{50}, K_i values and inhibition types for AR and SDH enzymes

Compounds	IC_{50}		K_i		Inhibition Type	
	AR	SDH	AR	SDH	AR	SDH
Cefazolin	14.74 mM	Activated	15.38 ±4.86	-	Uncompetitive	-
Ceftriaxone	28.75 µM	NI*	52.61 ± 11	-	Uncompetitive	-
Cefuroxime	5.65 mM	Activated	3.210 ± 0.3	-	Noncompetitive	-
Vancomycin	0.27 mM	NI*	0.106 ± 0.01	-	Competitive	-
Amikacin	9.80 mM	2.4 mM	7.540 ± 1.3	2.41 ± 0.3	Noncompetitive	Noncompetitive
Gentamicin	69.0 mM	38.5 mM	52.82 ±12.7	19.91±4.3	Uncompetitive	Uncompetitive
Cefotaxime	1.84 mM	NI*	2 ± 0.2	-	Noncompetitive	-
Ampicillin	4.96 mM	2.62 mM	2.72 ± 0.7	2.06 ±0.2	Noncompetitive	Noncompetitive
Ceftazidime	1.13 mM	3.76 mM	1.39 ± 0.1	ND*	Noncompetitive	-
Chloramphenicol	3.06 mM	NI*	3.07 ± 1.3	-	Uncompetitive	-
Lincomycin	345 mM	72 mM	ND**	40.33 ±5.1	ND**	Competitive
Metronidazole	58.4 µM	Activated	ND**	-	ND**	-
Ciprofloxacin	0.12 mM	NI*	ND**	-	ND**	-
Tylosin	49.3 µM	NI*	ND**	-	ND**	-

*No Inhibition

**Not Detected

both in vivo and in vitro activity was withdrawn from clinical tests as it developed hypersensitivity reactions due to the hydantoin ring in its structure [46, 47]. Imirestate, which is a fluorene analogue of sorbinil, was also withdrawn from clinical tests because of its toxic effect despite its satisfactory in vivo activity [48]. Tolrestat, which is a carboxylic acid derivative, was re-called worldwide because of its severe hepatic impairment and low in vivo effect [49]. Zopolrestat and Zenarestat were also withdrawn from clinical tests because of their damage to the kidneys and the liver [50]. On the other hand, it was shown that fidarestat, which is more effective and more selective than sorbinil that had a similar structure, provided beneficial clinical effects in diabetic neuropathy treatment with no important side effects. For this reason, fidarestat is one of the most promising drug candidates among ARIs for the time being [51-53]. Based on the abovementioned data, it is clear that it is very difficult to synthesize a new and effective aldose reductase inhibitor. The probability of new inhibitors synthesized to give positive results in clinical tests is also very low. For this reason, this study was conducted with the assumption that the current antibiotics, which have passed clinical tests successfully and have minimal effects on human health, maybe good inhibitors of aldose reductase and sorbitol dehydrogenase.

Table 4. Docking scores and binding energies of antibiotics with AR receptor. Docking scores and binding energies are given in kcal/mol.

Antibiotic	Docking Score	XP GScore	Glide evdw	Glide ecoul	Glide energy	Glide emodel
Chloramphenicol	-10.002	-10.002	-23.109	-13.613	-36.722	-61.419
Ceftriaxone	-9.934	-9.934	-47.539	-11.546	-59.085	-93.372
Cefotaxime	-8.848	-8.848	-43.366	-10.547	-53.913	-82.442
Cefuroxime	-8.231	-8.230	-34.993	-7.748	-42.742	-71.320
Ceftazidime	-8.055	-8.055	-39.697	-10.986	-50.683	-85.541
Cefazolin	-7.927	-7.927	-49.470	-12.16	-61.630	-100.438
Ampicillin	-7.834	-8.062	-34.596	-3.266	-37.862	-49.876
Lincomycin	-7.596	-7.606	-30.892	-4.943	-25.948	-30.792
Ciprofloxacin	-5.251	-7.930	-34.204	-6.863	-41.067	-58.777
Amikacin	-4.746	-4.809	-34.828	-2.81	-37.638	-44.630
Vancomycin	-4.271	-5.246	-38.922	-1.031	-36.953	-28.452
Metronidazole	-3.251	-3.261	-20.508	-0.055	-21.378	-26.983
Tylosin	-3.211	-3.232	-21.112	-0.064	-20.453	-25.798
Gentamicin	-3.199	-3.373	-16.936	-3.749	-20.685	-19.109

Table 5. Docking scores and binding energies of antibiotics with SDH receptor. Docking scores and binding energies are given in kcal/mol.

Antibiotic	Docking Score	XP Gscore	Glide evdw	Glide ecoul	Glide Energy	Glide emodel
Amikacin	-10.071	-10.135	-30.083	-24.415	-54.498	-66.025
Lincomycin	-6.754	-6.764	-32.15	-13.812	-45.962	-54.906
Gentamicin	-5.893	-6.068	-16.723	-24.861	-41.584	-50.266
Ceftazidime	-4.813	-4.813	-29.094	-9.211	-38.305	-54.38
Ampicillin	-4.545	-4.773	-29.798	-6.007	-35.805	-49.176

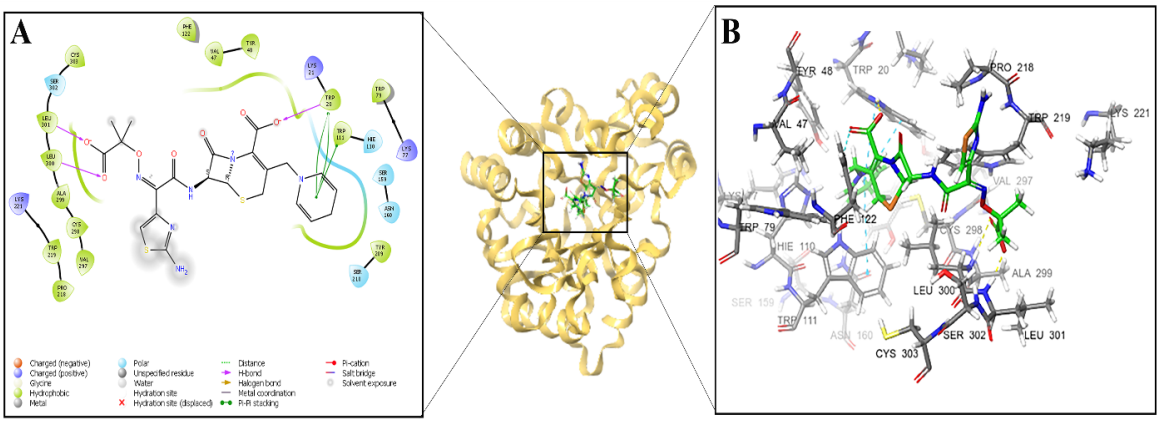

Figure 3. 2D ligand interaction diagram (A) and 3D detailed binding mode (B) of the ceftazidime antibiotic in the AR active site.

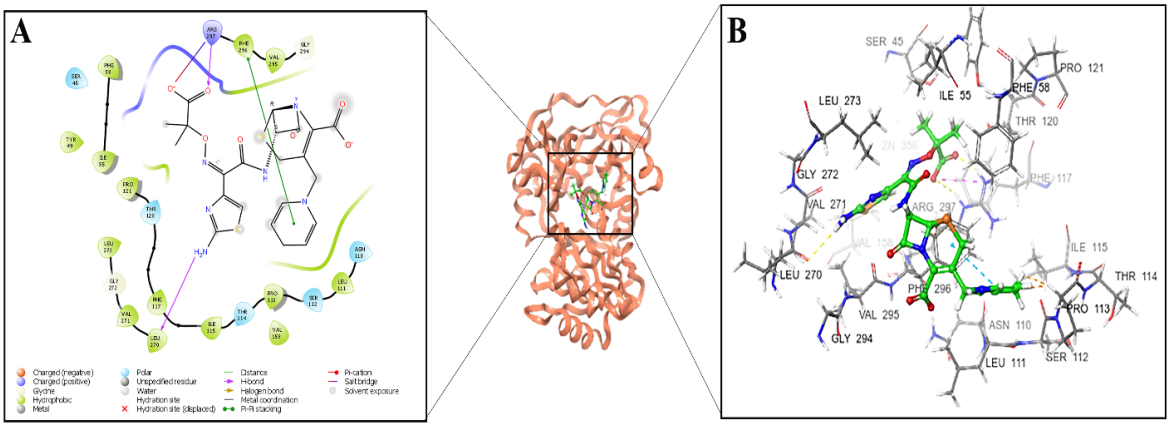

Figure 4. 2D ligand interaction diagram (A) and 3D detailed binding mode (B) of the ceftazidime antibiotic in the SDH active site.

In the present study, firstly, aldose reductase and sorbitol dehydrogenase enzymes were purified from the bovine kidney, and then the inhibition effects of some antibiotics on these enzymes were investigated. The results showed that the antibiotics that were examined are very good inhibitors for aldose reductase and sorbitol dehydrogenase. As shown in Table 3, the ampicillin and amikacin compounds inhibited both the aldose reductase and sorbitol dehydrogenase enzymes at very low concentrations.

The ceftriaxone compound showed the best inhibition effect with 28.75 µM on the AR enzyme. Ceftriaxone IC_{50} value was followed by tylosin and metronidazole with IC_{50} values of 49.28 µM and 58.42 µM, respectively. The lowest inhibitory effects were shown by lincomycin, gentamicin and cefazolin with IC_{50} values of 345 mM, 69 mM and 14.74 mM, respectively. The same compounds were examined on the SDH enzyme, and it was determined that the best inhibitory effect was shown with an IC_{50} value of 2.4 mM by the amikacin compound. Amikacin was followed by ampicillin and ceftazidime compounds with IC_{50} values of 2.62 mM and 3.76 mM, respectively. The lowest inhibition effect was shown by lincomycin and gentamicin with IC_{50} values of 72 mM and 38.5 mM, respectively. Lincomycin and gentamicin had very low inhibitory activities for both enzymes.

While the metronidazole compound inhibits the AR enzyme (IC_{50}=58.42 µM) strongly, it also activates the SDH enzyme. A similar situation is valid for the cefuroxime, and cefazolin compounds. The IC_{50} values of cefuroxime sodium and cefazolin for the AR enzyme are 5.65 mM and 14.74 mM, respectively. It is already known that the polyol pathway activity, which is increased by hyperglycemia, causes sorbitol accumulation in tissues resul-

ting in a slow-down of the production and conduction of nerve impulses in nerve cells, and eventually, diabetic complications like cataract. In this respect, it may be predicted that compounds like metronidazole, cefazolin and cefuroxime may reduce AR enzyme activity and inhibit sorbitol accumulation in tissues by increasing the SDH enzyme activity.

Docking studies for the AR receptor using Maestro 12.5 program of Schrödinger Molecular Modeling Suite have shown that antibiotics bind very well to the active site of the enzyme. It was determined that the ceftriaxone compound, which has a very good in vitro inhibitory effect on AR, makes hydrogen bonding with the amino acid residues TYR48, LYS77, HIE110, TRP111 and ASN160 in the anion binding pocket of the active site of the enzyme. In addition, it has also made π-π bonds with amino acid residues TRP20 and HIE110. Ceftazidime, which inhibits both the AR and the SDH enzymes, makes hydrogen bonding with the amino acids TRP20, LEU300 and LEU301 at the active site of the AR enzyme. It has also been found in π-π interaction with amino acids TRP20 and TRP111 (Figure 3). The binding of the ceftazidime compound to the 'specificity pocket'[54] at the active site of the AR enzyme indicates that the compound selectively inhibits the enzyme. The interaction of the ceftazidime antibiotic with the active site of the AR enzyme is similar to tolrestat, quercetin and epalrestat, which are very good inhibitors of the AR enzyme [55, 56]. According to these results, it can be concluded that the TRP20 and TRP111 amino acids play a very important role in the inhibition of the AR enzyme by antibiotics. Amikacin, which shows the best in vitro inhibitory effect on the SDH enzyme, has hydrogen-bonded with the amino acids LEU270, VAL271, GLY272, GLY274, and VAL295 in the active site of the enzyme. In addition, the compound made hydrogen bonding with residues LEU270 and ARG297, and π-π bond with PHE296 residue at the active site of the enzyme (Figure 4).

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