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The Influence of Some Nonsteroidal Anti-inflammatory Drugs on Metabolic Enzymes of Aldose Reductase, Sorbitol Dehydrogenase, and α-Glycosidase: a Perspective for Metabolic Disorders

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Abstract
Pain, as a sensible alarm signal of living organisms to avoid tissue damage, is a common and debilitating consequence of a lot of disorders and diseases. The management of chronic pain is particularly challenging. For pain treatment, many analgesic drugs are used for their therapeutic effects. In this study, some nonsteroidal anti-inflammatory drugs including etofenamate, meloxicam, diclofenac, and tenoxicam were tested against α-glycosidase from *Saccharomyces cerevisiae,* sorbitol dehydrogenase (SDH), and aldose reductase (AR) enzymes from sheep liver. Nonsteroidal anti-inflammatory drugs demonstrated useful inhibition properties against α-glycosidase, AR, and SDH enzymes. K_i values were found in the range of 11.93 ± 3.77–364.88 ± 40.01 μM for α-glycosidase, 3.36 ± 1.08μM–17.68 ± 3.39 mM for AR, and 1.68 ± 0.02 μM–30.98 ± 14.31 mM for SDH. They can be selective drugs as antidiabetic agents, because of their inhibitory properties against SDH, α-glycosidase, and AR enzymes.

Keywords Nonsteroidal anti-inflammatory drugs · Enzyme inhibition · α-Glycosidase · Aldose reductase · Sorbitol dehydrogenase

Introduction

Pain is a widespread disorder and an increasing result of many various diseases. Nonsteroidal anti-inflammatory drugs are used to treat intractable pain and clinical management

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of pain [1–3]. Tenoxicam is the well-established member of class nonsteroidal anti-inflammatory drugs. It is commonly employed in several toothaches, musculoskeletal disorders, arthritis, and symptomatic and dysmenorrheal relief of inflammation and pain [4]. Meloxicam is derived from oxicam which is a class of enolic acids. It exhibited a selective cyclooxygenase inhibitor property. It has analgesic and anti-inflammatory effects and showed a low level of gastrointestinal effects [5]. Diclofenac is used as a non-prescription, anti-inflammatory drug in animal and human health [6]. Etofenamate is used for the treatment of muscular and joint pain. Etofenamate acts by inhibiting cyclooxygenase, an enzyme which plays a role in prostaglandin synthesis, and has anti-inflammatory and analgesic properties [7].

Diabetes mellitus is a chronic and common metabolic disease characterized by abnormal glucose metabolism [8, 9]. In the tissues such as retina, nerves, kidney, and lens, a significant glucose flow is induced along the polyol pathway (PP) at high blood glucose levels [10]. The PP has a significant role in some diabetes complications including retinopathy, cardiovascular disease, neuropathy, cataract, and nephropathy [11].

Aldose reductase (AR) is the rate-determining enzyme of PP which catalyzes the conversion of glucose to sorbitol when the glucose quantity increases in the cell (Fig. 1). It is well known as an essential goal for therapeutic intervention to treat and also prevent diabetic complications [12]. Sorbitol dehydrogenase (SDH) is the second enzyme of the PP. The enzyme uses NAD^+ as a coenzyme and is a member of the oxidoreductase enzyme family. It is a homotetrameric enzyme which plays a vital role in the conversion of sorbitol to fructose in PP [13]. This metabolic pathway is very crucial for many tissues including sperm cells, placenta, kidney, retina, lens, and liver. Sorbitol accumulates within the cells under the hyperglycemic conditions with the absence of SDH [14].

One healing factor to therapy diabetes is to reduce the absorption of the glucose molecule via some enzyme's inhibition such as α-amylase and α-glycosidase, in the digestive cells [15, 16]. α-Glycosidase is an exo-kind carbohydrase that is extensively distributed in animal tissues, plants, and microorganisms, which catalyzes the release of α-glucose from the non-reducing terminal of the substrate [17, 18]. Inhibiting the α-glycosidase reduces the elevation of blood glucose after a carbohydrate diet. There are studies on α-glycosidase inhibitors (AGIs), like 1-deoxynojirimycin and nojirimycin of plants, acarbose, and voglibose of microorganisms, and as well as the effects of AGIs of wheat cores on blood glucose amounts after food uptake [19, 20].

In the present study, we focused on the purification of AR and SDH enzymes from sheep liver. Further, it also deals with the behavior of some nonsteroidal anti-inflammatory drugs as inhibitors against diabetes mellitus–related enzymes.

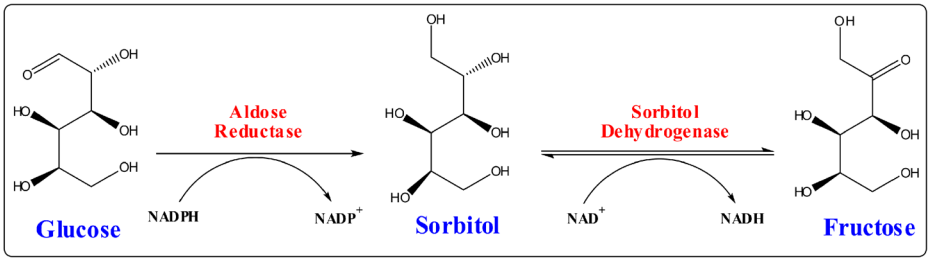

Fig. 1 The polyol pathway consists of aldose reductase and sorbitol dehydrogenase in the glucose metabolism

Materials and Methods

α-Glycosidase Activity

α-Glycosidase inhibitory effect of some nonsteroidal anti-inflammatory drugs was evaluated using the *p*-nitrophenyl-D-glycopyranoside (p-NPG) substrate realized according to the previous procedure [21]. Firstly, an aliquot of phosphate buffer (75 μL, pH 7.4) was added to 20 μL of the α-glycosidase solution, which was prepared in the same phosphate buffer solution (0.15 U/mL). Then, the mixture was pre-incubated at 37 °C for 10 min before adding the p-NPG (50 μL), which was prepared in phosphate buffer (5 mM, pH 7.4). Finally, the last solution was incubated at 37 °C. The absorbances of the mixture were spectrophotometrically recorded at 405 nm. One α-glycosidase unit is known as the amount of α-glycosidase, which catalyzes the hydrolysis of 1.0 mol substrate per minute at physiological pH (pH 7.4).

AR and SDH Activities

The AR activity was spectrophotometrically measured considering the decrease of NADPH at 340 nm using DL-glyceraldehyde [22]. On the other hand, the SDH activity was spectrophotometrically measured with 50 mM Glycine/NaOH in alkaline medium (pH 10.0) using substrates of NAD^+ (470 mM) and sorbitol (10 mM). The SDH activity was performed depending on the amount of NAD^+, which was recorded at 340 nm [23].

Purification of AR and SDH

For the first step of AR purification, the $(NH_4)_2SO_4$ precipitation was realized at a percent saturation of 40–80%. Then, precipitate dissolved with minimum phosphate buffer (10 mM, pH 7.4) and dialyzed in the same phosphate buffer containing β-mercaptoethanol (5 mM) at 4 °C. After this step, the dialyzed enzyme sample was loaded to the DE-52 cellulose ion exchange column. Proteins were eluted using NaCl gradient. Then, the fractions, which have activity, were loaded on the Sephadex G-100 ion exchange column and equilibrated with 10 mM phosphate buffer (pH 7.4). The AR activity obtained from Sephadex G-100 column fractions was spectrophotometrically recorded at 340 nm. The 2′5′-ADP Sepharose-4B affinity column was equilibrated with phosphate buffer (10 mM). The fractions from gel filtration chromatography were loaded into the affinity column. The enzyme was eluted by an increasing linear gradient of NaCl (0.25–2.5 mM) [24]. For SDH purification, DEAE-Sephadex and CM-Sephadex C50 columns chromatography methods were used according to the previous study [13].

Protein Determination

The protein quantity in fractions, which were obtained during all chromatographic methods, was measured according to the Bradford method using Coomassie Brilliant Blue G-250, which is commonly used for staining proteins in biochemistry studies [25].

Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was used for the determination of purity degree of the enzyme after the enzyme purification steps [26, 27]. Ten-

microgram samples were used for electrophoresis. The polyacrylamide concentration was 3% (*w*/*v*) for the stacking gel, 8% for the separating gel. The SDS-PAGE process was carried out according to the previous studies [26–29]. The gel was visualized with the silver staining method. The standard protein marker was purchased from Thermo Fisher Scientific Inc.

α-Glycosidase, AR, and SDH Inhibition Assays

In order to record the effect of some nonsteroidal anti-inflammatory drugs on α-glycosidase, AR, and SDH, various nonsteroidal anti-inflammatory drug concentrations were added into the reaction tube. For the preparation of the control sample, AR activity was measured in the absence of nonsteroidal anti-inflammatory drugs. The half maximal inhibitory activity (IC_{50}) was calculated from activity (%) versus some nonsteroidal anti-inflammatory drug concentration plots according to previous studies [30, 31]. Also, for the determination of the inhibition constant (K_i), diverse substrates (*p*-NPG, sorbitol, and DL-glyceraldehyde) concentrations were used. Drug solution was added into the reaction mixture, resulting in three different fixed concentrations of inhibitor utilized for the calculation of V_{max} and other inhibition parameters like K_m or K_i. The K_i values were calculated from Lineweaver-Burk graphs [32].

Results and Discussion

The PP consists of two enzyme members, which are AR and SDH enzymes. AR belongs to aldo-keto reductase superfamily and plays a vital role in the PP. It catalyzes the conversion of monosaccharide of glucose to sorbitol, which is known as glucitol and sugar alcohol, when the glucose concentration increases in the cell [33]. On the other hand, SDH oxidizes sorbitol to fructose. Also, it can be formed via glucose reduction by changing the aldehyde group in glucose to a hydroxyl group (–OH). In the case of accumulation or overproduction of sorbitol monosaccharides, osmotic stress occurs in the living cells [34]. Indeed, this condition may trigger the progression of different diabetic complications. Hence, determining the inhibition effects of chemicals or drugs on PP enzymes is very significant [35]. The PP is the most promising and the most commonly studied among these pathways. Excessive glucose is metabolized via the PP. AR is the first and crucial enzyme in the PP and converts glucose to sorbitol. Then, SDH reduces sorbitol to fructose [36]. Recent studies reported that raised substrate flux via PP related to an increased cytosolic NADH/NAD^+. Inhibition of AR decreased the ratio of NADH/NAD^+; also, PP metabolites fructose and sorbitol levels. The decrease in the cytosolic redox state was accompanied by raised glucose metabolism. Hence, AR and SDH inhibitors are becoming potential drugs. Many compounds are improved as inhibitors in the past few decades. However, many lead to the success on the market or failed to be advanced through the late stage of clinical trials owing it to their side effects or reduced efficacy, which were often related to a deficiency of AR selectivity [37, 38].

In the current study, AR was purified with a specific activity of 3.00 EU mg^{-1} proteins, 214.29-fold and yield of 0.88% from sheep liver (Table 1). SDH was purified with a specific activity of 0.23 EU mg^{-1} proteins, 23.0-fold and yield of 2.22% from sheep liver (Table 2). The purity of the enzymes was checked by SDS-PAGE method. As seen in Fig. 2, the molecular weight was found approximately 38 kDa for AR and 39 kDa for SDH. The inhibitory effects of nonsteroidal anti-inflammatory drugs including etofenamate, meloxicam, tenoxicam, and diclofenac (Fig. 3) on the AR, SDH, and α-glycosidase were determined. Both

the inhibition parameters (IC_{50} and K_i values) of the analgesic drugs were determined via activity (%) (nonsteroidal anti-inflammatory drug) and Lineweaver-Burk graphs (1/V-1/[S]), respectively.

For AR as seen in Table 3, the K_i values were found to be > 1000 μM for etofenamate, 3.36 ± 1.08 μM for meloxicam, 898.01 ± 9.03 μM for diclofenac, and 41.82 ± 3.54 μM for tenoxicam. Additionally, all compounds were exhibited noncompetitive inhibitions. In other words, there is no competition between the substrate and nonsteroidal anti-inflammatory drugs for binding to the active site of AR enzyme. For SDH, the IC_{50} values were found to be > 1000, 0.01, and 0.013 μM for etofenamate, meloxicam, and diclofenac, respectively. K_i constants were calculated as > 1000, 2.48 ± 1.41, and 1.68 ± 0.02 μM for etofenamate, meloxicam, and diclofenac, respectively (Table 3). Etofenamate and meloxicam were exhibited noncompetitive inhibition, while diclofenac was exhibited uncompetitive inhibition. However, tenoxicam did not show the inhibition effect on the SDH enzyme.

Metabolism involves all the necessary series of reactions that permit the movement of living things, and these series are called metabolic pathways. In the organism, metabolic pathways are the actual and functional units for the metabolic network [39]. In this way, a disorder in any path generates an increasingly snowball effect in all metabolism. In living organisms, enzymes have a crucial role and catalyze all chemical reactions in living metabolism. Many drugs and chemicals impact metabolism at low concentrations by increasing or decreasing regular enzyme activity. Primarily, they inhibit specific enzymes with critical function, which are important drug targets [40, 41]. Researchers had many studies about interactions between different enzymes and drugs. For instance, the inhibition effects of some epileptic drugs were determined on paraoxonase activity [42, 43]. In another study, the SDH enzyme was purified from sheep liver, and the effects of some anti-neoplastic drugs were studied [13].

There are many studies about drugs or chemical effects on PP enzymes especially AR in literature. For example, cefuroxime, ceftazidime, cefoperazone, ceftriaxone, and cefazolin were selected for investigating inhibition effects against SDH and AR activity. They showed that all compounds inhibited AR, but SDH was inhibited only by cefuroxime [44]. In another

Table 1 Purification steps of aldose reductase (AR) from sheep liver

Purification steps	Enzyme activity (EU/mL)	Total volume (mL)	Protein (mg/mL)	Total protein (mg)	Total activity (EU)	Specific activity (EU/mg)	Yield (%)	Purification fold
Homogenate	0.38	27	27.26	736.02	10.26	0.014	100	1
Ammonium sulfate precipitation and dialysis	0.41	22	19.25	423.50	9.02	0.021	87.91	1.50
DE-52 cellulose anion exchange chromatography	0.26	18	9.43	169.74	4.68	0.028	45.61	2.00
Sephadex G-100 gel filtration chromatography	0.11	9	1.11	9.99	0.99	0.10	9.65	7.14
2′5′ ADP Sepharose 4B affinity chromatography	0.03	3	0.01	0.03	0.09	3.00	0.88	214.29

Table 2 Purification steps of sorbitol dehydrogenase (SDH) enzyme from sheep liver

Purification steps	Enzyme activity (EU/mL)	Total volume (mL)	Protein (mg/mL)	Total protein (mg)	Total activity (EU)	Specific activity (EU/mg)	Yield (%)	Purification fold
Homogenate	0.27	25	27.26	681.50	6.75	0.01	100	1
Ammonium sulfate precipitation and dialysis	0.25	22	21.45	471.90	5.50	0.012	81.48	1.2
DE-52 cellulose anion exchange chromatography	0.17	15	8.23	123.45	2.55	0.021	37.38	2.10
CM cellulose anion exchange chromatography	0.13	10	2.85	28.50	1.30	0.046	19.26	4.60
Sephadex G-100 gel filtration chromatography	0.05	3	0.22	0.66	0.15	0.23	2.22	23.0

study, in vitro inhibition effects of sinapic acid, tannic acid, 4-hydroxybenzoic acid, chlorogenic acid, protocatechuic acid, p-coumaric acid, vanillic acid, α-resorcylic acid, ferulic acid, syringic acid, gallic acid, and 3-hydroxybenzoic acid as phenolic acids on purified rat

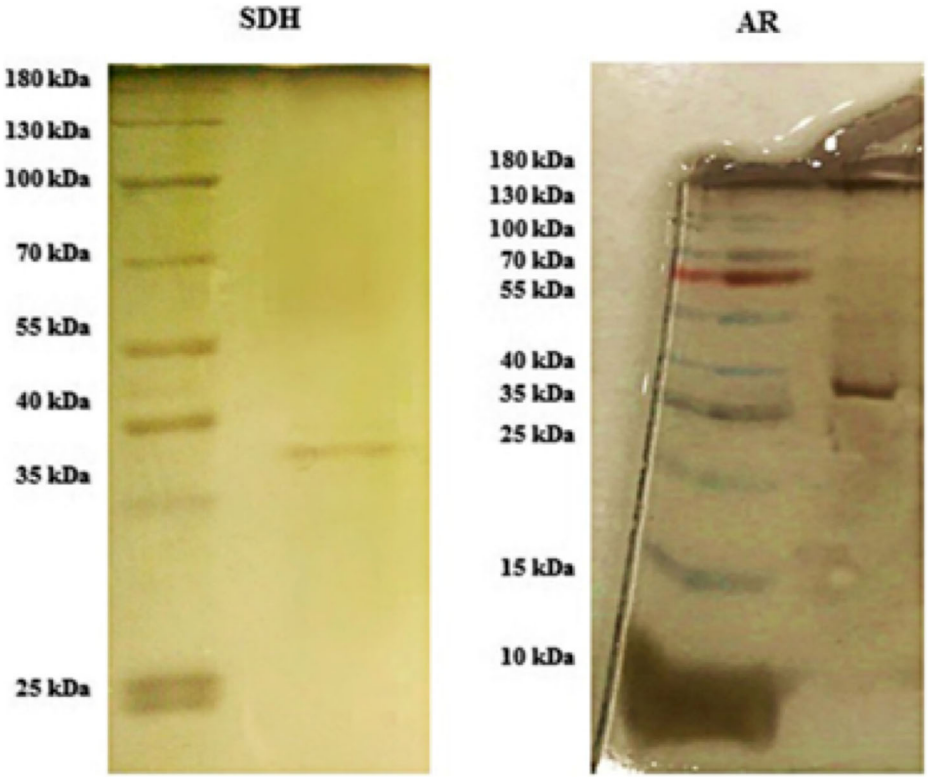

Fig. 2 SDS-PAGE analysis of SDH and AR to confirm the purity of the enzymes was carried out according to the Laemmle's method. Samples were used for the electrophoresis, and the protein bands were obtained with silver staining. The standard protein marker was purchased from Thermo Fisher Scientific Inc.

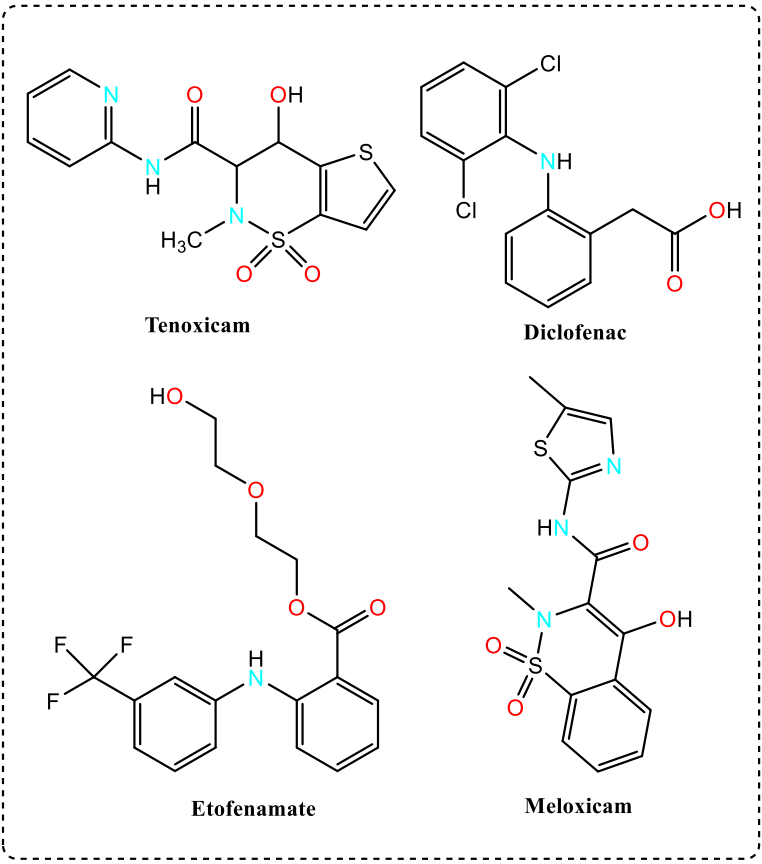

Fig. 3 Chemical structures of active ingredients of some nonsteroidal anti-inflammatory drugs

kidney AR were investigated. K_i and IC_{50} values showed that chlorogenic and tannic acids were selective inhibitors against the AR enzyme [24].

Additionally, in this work, it was determined the α-glycosidase inhibition effects of nonsteroidal anti-inflammatory drugs (Fig. 3). The used drugs had IC_{50} values in the range of 8.18–408.61 μM. IC_{50} values of some nonsteroidal anti-inflammatory drugs exhibited the following order: meloxicam (8.18 μM; r^2, 0.9730) < etofenamate (87.35 μM; r^2, 0.9818) <

Table 3 Inhibition effects of some nonsteroidal anti-inflammatory drugs including etofenamate, meloxicam, diclofenac, and tenoxicam on aldose reductase (AR), sorbitol dehydrogenase (SDH), and α-glycosidase (α-Gly) enzymes

Drug names	AR inhibition (μM)		SDH inhibition (μM)		α-Gly inhibition (μM)	
	IC_{50}	K_i	IC_{50}	K_i	IC_{50}	K_i
Etofenamate	7.87*	17.68 ± 3.39*	0.014*	30.98 ± 14.31*	87.35	62.07 ± 14.83
Meloxicam	1.29	3.36 ± 1.08	0.010	2.48 ± 1.01	8.18	11.93 ± 3.77
Diclofenac	783.00	898.01 ± 9.03	0.013	1.68 ± 0.02	408.61	364.88 ± 40.01
Tenoxicam	18.89	41.82 ± 3.54	–	–	103.63	88.52 ± 11.62

*Figures in mM

tenoxicam (103.63 μM; r^2, 0.9473) < diclofenac (408.61 μM; r^2, 0.9792). On the other hand, they demonstrated K_i values in the range of 11.93 ± 3.77–364.881 ± 40.01 μM (Table 3). K_i values of these drugs exhibited the following order: meloxicam (11.93 ± 3.77 μM) < etofenamate (62.07 ± 14.83) < tenoxicam (88.52 ± 11.62 μM) < diclofenac (364.881 ± 40.01 μM) (Table 3).

Recently, efforts for the extension of a novel set of AGIs have focused generally on sugar mimics, such as disaccharides, iminosugars, carbasugars, and thiosugars [45, 46]. However, multiple losses exist for this process, including complex stereochemistry, or low natural abundance, reduced activity, which creates them challenging to approach synthetically [47]. A more modern procedure is to evaluate nonsugar AGIs. They have more considerable thousands of times affinity with carbohydrate-binding sites than polysaccharides and oligosaccharides [48]. The oligosaccharides and polysaccharides cannot be transformed into monosaccharides and disaccharides that the bodies can absorb [49]. Thus, α-amylase and α-glycosidase inhibitors are essential drugs to inhibit the assimilation of carbohydrate molecules in the intestines, which can be utilized, to therapy diabetes mellitus type 2 (T2DM), and disordered glucose tolerance [50]. Therefore, these hypoglycemic factors are combined with other drugs or applied to patients with early diabetes. Miglitol and acarbose are AGIs utilized to clinically treat patients with T2DM [51].

Conclusions

Nonsteroidal anti-inflammatory drugs which relieve pain and are extensively employed in virtually all branches of medicine which are used in the present study recorded powerful inhibition effects against some metabolic enzymes including α-glycosidase, AR, and SDH. In this work, micromolar IC_{50} and K_i values were obtained for all studied drugs enzymes, while etofenamate showed millimolar levels were observed for AR and SDH. Thus, these drugs can be a selective inhibitor of AR, SDH, and α-glycosidase enzymes. AGIs recorded as therapeutic agents for the treatment of some metabolic disorders including obesity, diabetes mellitus, and hyperglycemia. AGIs and ARIs are also recognized to be promising as antiviral and antitumor effects that interfere with the biosynthesis of N-linked oligosaccharide chains.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflicts of interest.

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