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# RELATIONSHIP BETWEEN ALDOSE REDUCTASE AND SUPEROXIDE DISMUTASE INHIBITION CAPACITIES OF INDOLE-BASED ANALOGS OF MELATONIN DERIVATIVES

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*Abstract* — Aldose reductase (AR) has been implicated in the etiology of diabetic complications. Under diabetic conditions, the elevated vascular glucose level causes an increased flux through the polyol pathway, which induces functional and morphological changes associated with secondary diabetic complications such as cataract, neuropathy, and nephropathy. Oxidative stress, antioxidants, and the polyol pathway have recently been found to be linked in pathological states. A large number of structurally different compounds have been studied as potent in vitro AR inhibitors (ARIs). However, with few exceptions, these compounds did not show clinical benefit, and some even produced serious side effects. In view of the ARI activity of certain indole derivative compounds and antioxidant properties of melatonin, we investigated some indole-based analogs of melatonin derivatives. Antioxidant and ARI activity tests were applied to nine indole derivatives that are substituted at the third and fifth positions. Also, the relationship between ARI and antioxidant enzyme activity is discussed.

*Key words*: Aldose reductase, antioxidant, diabetes mellitus, indole, melatonin

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## INTRODUCTION

Reduction of glucose by the enzyme aldose reductase (AR) leads to the formation of sorbitol, which, in some tissues, is further oxidized to fructose upon sorbitol dehydrogenase-catalyzed oxidation (Suzen et al., 2003). Aldose reductase of the polyol metabolic pathway, apart from its role as a sorbitol producer and detoxifier of toxic aldehydes, osmoregulator in the lens and kidney, and regulator of sperm maturation, has been implicated in the etiology of long-term diabetic complications (Gabbay, 1975; Gui et al., 1995). This enzyme is involved in many pathological processes that have become major threats to human health. Such pathologies include a number of cardiac disorders, inflammation, mood disorders, renal insufficiency, and ovarian abnormalities (Oates, 2008; Alexiou et al., 2009). Oxidative stress and the polyol pathway have recently been found to be linked in pathological states (Suzen, 2006, 2007; Suzen et al., 2007).

The important antioxidant enzyme superoxide dismutase (SOD) catalytically removes the superoxide radical and protects organisms from oxidative damage. Superoxide is among the most abundant reactive oxygen species (ROS) produced by the mitochondria, and is involved in cellular signaling pathways. Superoxide and other ROS can damage cellular macromolecules and enhance the levels of oxidative damage. Superoxide dismutase catalyzes the breakdown of superoxide into hydrogen peroxide and water and is therefore one of the central regulators of ROS levels (Srivastava et al., 2005). Reactive oxygen species such as superoxide, hydrogen peroxide, and hydroxyl radicals are constantly being generated intracellularly in aerobic organisms and may play a role in the pathogenesis of diabetes mellitus, ischemia-reperfusion injury, cancer, inflammatory disease, and atherosclerosis, as well as in cell death and tissue injury (Thomas et al., 2000; Yildirim et al., 2003, 2007). Recent studies suggest that glucose may be an incidental substrate of AR, which appe-

ars to be more adept in catalyzing the reduction of a wide range of aldehydes generated from lipid peroxidation. Moreover, inhibition of the enzyme has been shown to increase inflammation-induced vascular oxidative stress and prevent myocardial protection associated with the late phase of ischemic preconditioning (Satih et al., 2005).

Basically, the inhibitors of AR mostly belong to two chemical classes, spirosuccinamide/hydantoins and carboxylic acids. Each class has its own drawbacks regarding selectivity, *in vivo* potency, and human safety; as a result, the pathogenic role of AR in diabetic complications remains controversial (Sun et al., 2006).

Miyomoto (2002) found that an indole acetic acid derivative showed more than 40% inhibition of AR at a concentration of 15 μg/mL. After a subsequent optimization study based on the predicted docking mode, an approximately 20-fold increase in inhibitory activity of the indole derivative was achieved.

The designers of 4-oxo-4-(4-hydroxyindole) butanoic acid was took into account the CoMSIA field and the binding mode derived by FlexX docking (Sun et al., 2003). This rationally designed compound exhibits an ALR2 inhibition with an IC(50) value of 7.4 μM, which compares favorably to that of a well-known ALR2 inhibitor, tolrestat [IC(50) = 16 μM].

Cyano(2-oxo-2,3-dihydroindol-3-yl)acetic acid derivatives were synthesized and tested as a novel class of aldose reductase (ALR2) inhibitors by Da Settimo et al. (2003). Introduction of a halogen and a lipophilic group in the 5- and 1-positions, respectively, of the indole nucleus displayed the highest activity [IC(50) 0.075 μM], very close to that of tolrestat [IC(50) 0.046 μM]. Also, carboxymethylated hexa/tetrahydropyridoindoles structurally based on the antioxidant drug stobadine were presented as novel ARIs capable of antioxidant activity by Stefek et al. (2008). A 5-(3'-indolal)-2-thiohydantoin derivative was found to be effective in reducing the enzyme's activity compared to a corresponding well-known AR inhibitor (Buyukbingol et al., 1994).

A large number of structurally different compounds have been studied as potent *in vitro* ARIs (Bozdağ-Dündar et al., 2007, 2008; Suzen et al., 2007; Daş-Evcimen et al., 2008). However, with few exceptions, these compounds did not show clinical benefit. A compound with effective inhibition of AR and oxidative stress would be a most promising drug for the prevention of secondary diabetic complications (Lim et al., 2001).

Indole derivatives such as melatonin constitute an important class of therapeutical agents. In view of the ARI activity of indole derivative compounds and antioxidant properties of melatonin (Karbownik et al., 2001), we have been studying antioxidant and AR inhibition activities of indole derivatives for more than a decade (Ates-Alagoz et al., 2006; Suzen et al., 2006; Gurkok et al., 2009). Our results indicate that indole base analogs of melatonin derivatives have promising activities. In the light of previous results, the aim of this study was to investigate indole derivative compounds with these dual effects by examining structure-activity relationships with respect to inhibition of rat lens aldose reductase and antioxidant action. To this end, AR and SOD inhibitory capacities of nine indole derivatives were tested *in vitro*.

## MATERIAL AND METHODS

### *Chemicals*

The tested compounds were all purchased from Aldrich. All solvents and reagents were of reagent grade and were supplied by Sigma or Aldrich.

### *Materials*

Thirty bovine eyes were obtained from a slaughterhouse. They were frozen at -80°C. Their lenses were taken out for isolation of the enzymes AR and SOD.

### *Isolation of aldose reductase*

The enzyme AR was isolated by the method (Cerelli et al., 1986) described below. Pooled kidneys were thawed on ice and homogenized with three volumes of distilled water, followed by centrifugation at 10000g for 20 min. Saturated ammonium sulfate

was added to the supernatant to 40% saturation. The thick suspension was stirred for 15 min, then centrifuged at 10000g for 20 min. The inert protein left in the supernatant was removed by increasing the ammonium sulfate concentration to 50% saturation, followed by centrifuging the mixture at 10000g for 20 min. Aldose reductase was precipitated from the 50% saturated solution by adding powdered ammonium sulfate to 75% saturation and was recovered by centrifugation at 10000g for 20 min. Protein concentration was measured by the method of Bradford (1976) using bovine serum as the standard.

*Determination of aldose reductase activity*

Aldose reductase activity of the freshly prepared supernatant was assayed spectrophotometrically by determining the decrease in NADPH concentration at 340 nm using a UV-1700 visible spectrophotometer (Shimatzu) (Cerelli et al., 1986) DL-glyceraldehyde was used as the substrate. The enzyme was dissolved in 10 ml of 0.05 M NaCI solution. A 6.40-mg quantity of protein was added to a quartz cuvette containing 0.1 ml of phosphate buffer (0.067 M, pH 6.2), 0.1ml of NADPH ($2 \times 10^{-5}$ M final concentration), 0.1 ml of the test drug ($10^{-4}$ M solutions prepared in 50% DMF-methanol), and 2.4 ml of distilled water to obtain 2.9 ml of solution. The reaction was started by adding 0.1 ml of DL-glyceraldehyde ($5 \times 10^{-5}$ M final concentration ) to the cuvette, and decrease of NADPH concentration was recorded at 340 nm for 5 min at 37°C. Readings were taken at intervals in the periods when changes in absorbance were linear.

*Tissue sample preparation for SOD activity*

Lens tissues were homogenized in three volumes of 50 mM phosphate buffer (pH 7.4) at 4°C for 30 sec (2 x 15 sec with a 15 sec cooling interval) at 2000 rpm using a Teflon glass pestle with a Heidolph homogenizer. Cell debris was removed by centrifugation at 4°C and 10000g for 20 min. The resultant supernatant was used for measurement of SOD activity.

*Determination of SOD activity*

A simple assay system (Kostyuk et al., 1989) based on the inhibitory effects of SOD on spontaneous oxidation of quercetin was used. Oxidation of quercetin was previously shown to be a free radical chain reaction involving superoxide and hence inhibitable by SOD. The degree of inhibition of quercetin oxidation was a function of SOD concentration. This reaction proved to be a very useful tool for rapid and highly sensitive measurement of SOD in crude tissue extracts and other biological samples.

The rate of quercetin oxidation in 0.016 M phosphate buffer (pH 9.2) was determined by observing absorbance changes at 406 nm spectrophotometrically. The incubation medium contained 8.96 mg of protein, $1.405 \times 10^{-5}$ M quercetin, and 0.014 M phosphate buffer in 3 ml. One unit is the amount of SOD required to inhibit the initial rate of quercetin oxidation by 50%.

## RESULTS AND DISCUSSION

We have studied the possible effects of nine indole-based melatonin derivatives on AR and SOD activities. All experiments were performed in triplicate. The results are shown in Table 1. According to the results, the highest inhibitory activity on AR was observed with compound 8 (71%). Compounds 9 and 6 showed slight inhibitory activity, 25 and 21%, respectively. Other compounds did not show significant AR inhibitory activity (Table 1). On the other hand, compounds 2, 7, 8, and 9 enhanced SOD activity only slightly, but did not inhibit it. It was found that compound 6 inhibited SOD.

Due to the limited number of available drugs for the treatment of diabetic complications, a number of reasonable approaches for the discovery of ARIs have been taken. It is a very well-known fact that glucose concentrations are often elevated in diabetics, and AR has long been believed to be responsible for diabetic complications involving a number of organs. Many ARIs have been developed as drug candidates, but virtually all have failed, although some are commercially available in several countries.

Oxidative stress, antioxidants, and the polyol pathway are known to be linked in pathological states; changes in the polyol pathway can change the redox status of cells, thereby altering the activities of

**Table 1.** Inhibition of AR and SOD and activation of SOD by indole derivatives.

| Compound | AR<br>% Inhibition | SOD<br>% Inhibition | SOD<br>% Activity |
|---|---|---|---|
| 1 | 14.92 ± 1.75 | 11.28 ± 8.20 | |
| 2 | 5.05 ± 2.65 | | 2.36 ± 9.83 |
| 3 | 1.51 ± 1.25 | 0.00 ± 10.02 | |
| 4 | 0.00 ± 0.00 | 20.80 ± 2.15 | |
| 5 | 8.64 ± 1.93 | 12.82 ± 9.86 | |
| 6 | 21.28 ± 0.64 | 28.47 ± 10.27 | |
| 7 | 14.96 ± 2.93 | | 10.41 ± 4.63 |
| 8 | 71.38 ± 0.58 | | 4.62 ± 3.56 |
| 9 | 25.17 ± 10.35 | | 1.19 ± 6.28 |

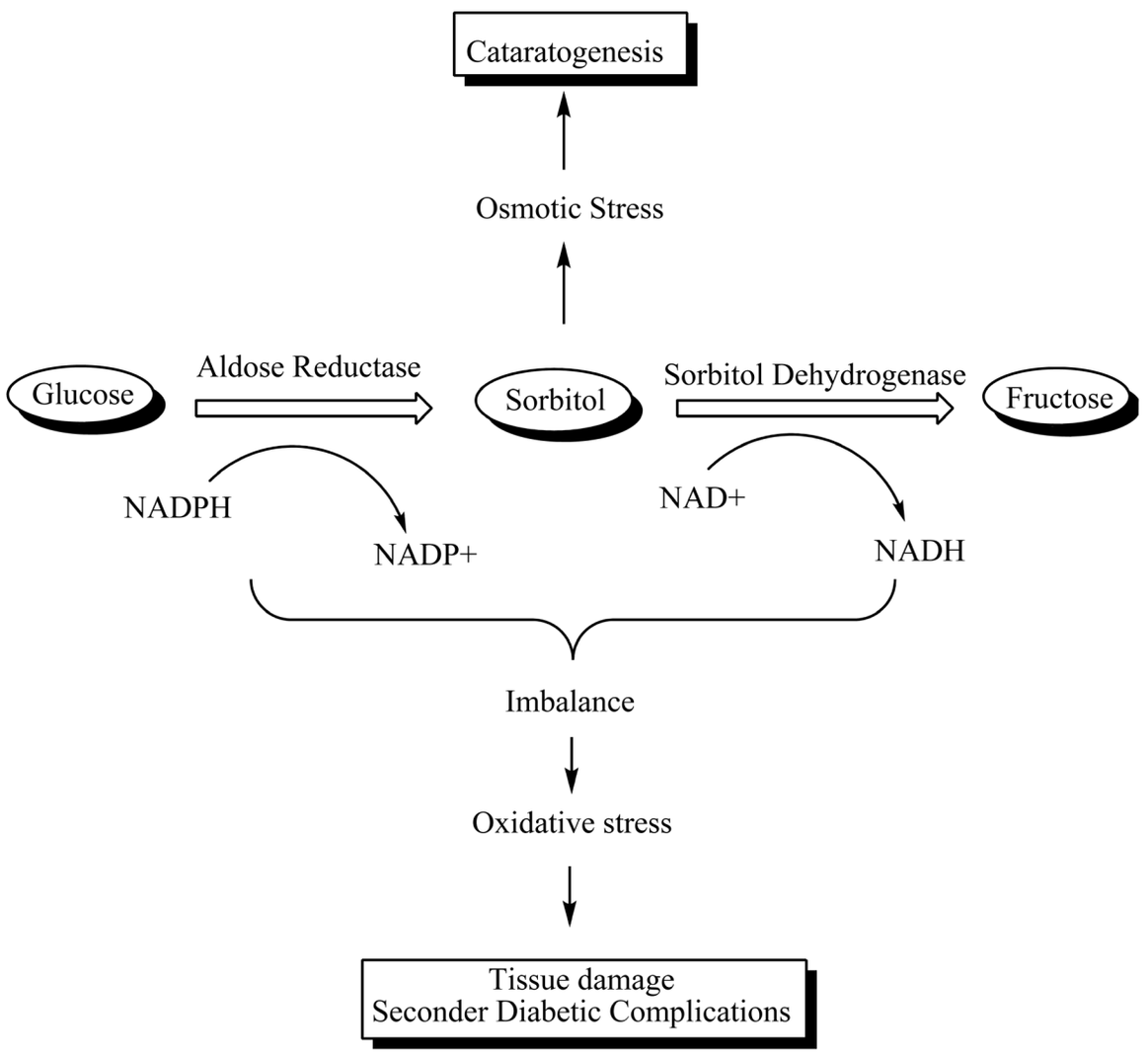


**Fig. 1.** Involvement of polyol pathway in diabetic complications. Adapted from Srivasta et al. (2005).

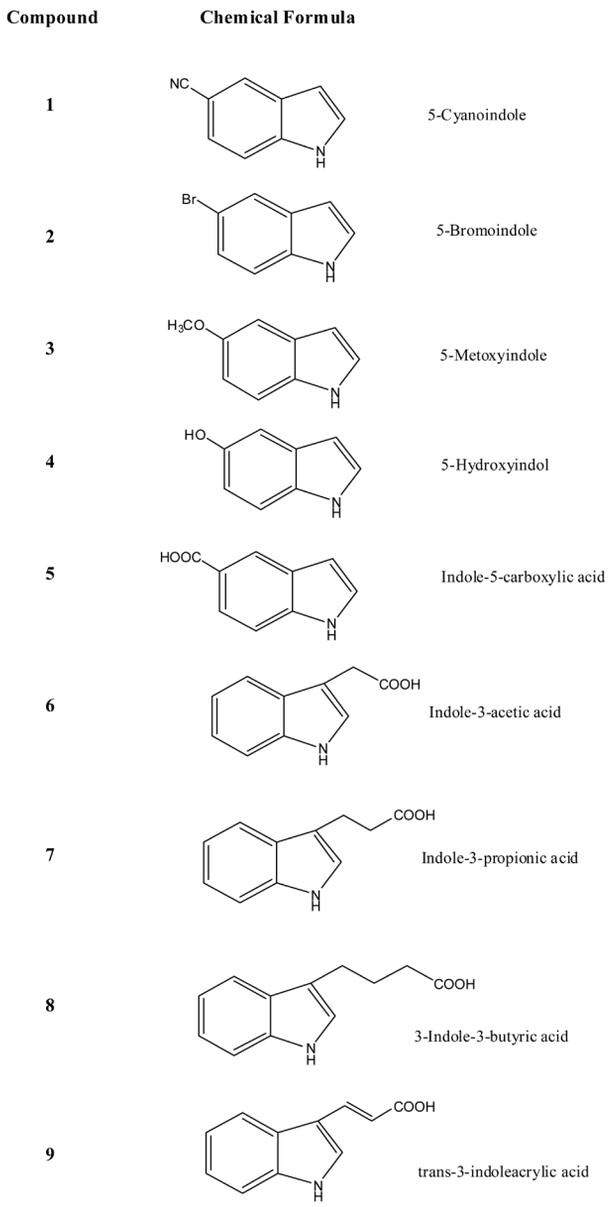


**Fig. 2.** Investigated indole-based analogs of melatonin derivatives.

oxidative enzymes (Thomas et al., 2000). The relationship between AR activity and oxidative stress in tissue sites for diabetic complications remains controversial. The result obtained for compound 8 represents a promising step for further studies of AR inhibitory drug development.

Owing to the pharmacophoric need of ALR-2 (Lee et al., 1998; Schlitzer et al., 2001) for an acidic proton, most ARIs contain an acetic acid moiety or *N*-unsubstituted cyclic imides. Introduction of bioisosteric groups to the indole ring may represent another way to overcome unfavorable partitioning of acidic ARIs (Nikolaou et al., 2004; Rakowitz et al., 2005; Gurkok et al., 2009). Oxidative stress and involvement of the polyol pathway in the etiology of diabetic complications have been identified (Baynes et al., 1999; Obrosova, 2005). Based on the principle that a bifunctional compound with joint antioxidant/aldose reductase inhibitory activities could be multifactorially beneficial, *N*-protected 1*H*-indole-5-carboxylic acid derivatives were proposed as potential ARIs (Nikolaou et al., 2002) earlier. In this study, compound 5 (indole-5-carboxylic acid) did not show noteworthy ARI or antioxidant activities.

Compound 8 (indole-3-propionic acid, IPA) has previously been identified in the plasma and cerebrospinal fluid of humans. This compound completely protected primary neurons and neuroblastoma cells against oxidative damage and death caused exposure to Aß, inhibition of superoxide dismutase, or treatment with hydrogen peroxide. In kinetic competition experiments using free radical-trapping agents, the capacity of IPA to scavenge hydroxyl radicals exceeded that of melatonin, an indoleamine considered to be the most potent naturally occurring scavenger of free radicals. In contrast to other antioxidants, IPA was not converted to reactive intermediates with pro-oxidant activity. These findings may have therapeutic applications in a broad range of clinical situations (Chyan et al., 1999). Melatonin and IPA completely prevented DNA damage in hamster kidneys. Karbownik et al. (2001) concluded that melatonin and IPA may be effective in protecting against DNA damage and, consequently, carcinogenesis.

The results of this study suggest that compound 8 may play an important role in antioxidant and ARI effects and would clearly have potential uses in the development of therapeutic or preventive agents for diabetic complications and oxidative stress-related diseases.

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