Differences in the susceptibility of various aldose reductases to inhibition. II. Peter F. Kador, Jin H. Kinoshita, William H. Tung, Leo T. Chylack, Jr.

The susceptibility of human lens aldose reductase (HLAR), human placental aldose reductase (HPAR), and rat lens aldose reductase (RLAR) to inhibition by 10 structurally diverse inhibitors is compared. Significant differences in the susceptibility of these enzymes was observed; however, no trends could be predicted. In general, HPAR aldose reductase (REAR) to inhibition by 10 structurally diverse inhibitors is compared. Significant differences in the susceptibility of these enzymes was observed; however, no trends could be predicted. In general, HPAR appeared to be less susceptible to inhibition than either HLAR or RLAR, with the susceptibility of HLAR being more similar to RLAR than to HPAR. These findings indicate that the evaluation of aldose reductase inhibitors for potential clinical use may require the use of human aldose reductase from the appropriate target tissue.

Aldose reductase (alditol: NADPH oxidoreductase, E.C. 1.1.1.21), an enzyme in the sorbitol pathway of glucose metabolism, catalyzes the reduction of aldose sugars to sugar alcohols. In the lens of galactosemic or diabetic animals the formation of excess sugar alcohols in lens fibers followed by the osmotic accumulation of water directly leads to cataract formation. Evidence also suggests aldose reductase to be involved in other diabetic complications, including neuropathy and retinopathy.

Generally, hexoses, including glucose, are poor substrates for aldose reductase; however, when their levels are elevated as in hyperglycemia or hypergalactosemia, significant polyol formation can occur. That aldose reductase plays a significant role in abnormal physiological conditions such as hyperglycemia makes the inhibition of aldose reductase an attractive potential treatment for diabetic complications. In the eye, aldose reductase inhibitors have been shown to delay cataract formation and aid corneal re-epithelialization of diabetic and galactosemic animals.

Currently, aldose reductase inhibitors are screened for potential inhibitory potency with enzymes from animal sources. Significant differences in the susceptibility of rat lens aldose reductase (RLAR) and human placental aldose reductase (HPAR) to inhibition have been previously noted.

Here, we extend these observations to human lens aldose reductase (HLAR) by comparing the susceptibility of RLAR, HPAR, and HLAR to inhibition by a series of structurally diverse compounds.

Materials and methods. RLAR and HPAR were prepared as previously described. Alternately, HPAR was prepared by homogenizing pla-centa with 2 to 3 parts of 0.1M Na,K-phosphate buffer, pH 6.8, containing 1 mM mercaptoethanol and 1 mM Nicotinamide adenosine dinucleotide phosphate (NADP). The homogenate was then centrifuged at 10,000 × g for 15 to 20 min, and the supernatant was fractionated with ammonium sulfate. The precipitate obtained from the 30% to 50% fraction by centrifugation at 45,000 × g for 10 min was redissolved in distilled water and stored at −78° C. This more stable enzyme preparation gave inhibitions identical with those obtained with purified enzyme. HPAR was prepared by homogenizing freshly excised lenses, deucleated with a 5 mm borer, in 0.055M Na,K-phosphate buffer, pH 6.8 (1.5 ml/lens). After centrifugation at 27,000 × g for 20 min, 0.4M lithium sulfate was added to the supernatant, and the enzyme preparation was immediately used.

Aldose reductase activity was spectrophotometrically assayed in a Gilford 2400-2 automated compensation spectrophotometer by determining the decrease in the concentration of NADPH at 340 nm. The reaction mixture contained 0.1M Na,K-phosphate buffer, pH 6.2, 0.104 mM NADPH, and 10 mM DL-glyceraldehyde in a total volume of 1.00 ml. The reference blank contained all the above compounds except glyceraldehyde. Appropriate blanks to correct for the nonspecific reduction of NADPH and the absorption of inhibitor were employed. Log dose-response curves were then constructed, and the concentration of inhibitor which was necessary for 50% inhibition of activity (IC50) was estimated from the least-square regression line of the log dose-response curve.

Compound 1 was obtained from Ayerst Laboratories, Montreal, Canada. Compound 2 was obtained from ICN-K&K Laboratories, Inc., Cleveland, Ohio. Compound 3 was obtained from Pfizer Central Research, Groton, Conn. Compounds 4 and 5 were synthesized as previously described. Compound 6 was a gift from Dr. G. P. Ellis of the Department of Chemistry, University of Wales Institute of Science and Technology, Cardiff, Wales. Compounds 7 to 10 were supplied by The Upjohn Co., Kalamazoo, Mich.

Results and discussion. The susceptibility of HLAR, HPAR, and RLAR to inhibition by the known structurally diverse, aldose reductase inhibitors (1) Alrestatin (AY-22,284, 1,3-dioxo-IH-benz[de]isoquinoline-23H) acetic acid; (2) querc-
citrin (2-(3,4-dihydroxyphenyl)-3-O-rhamnosyl-5,7-dihydroxy-4-oxo-4H-chromen), and (3) Sorbinil (S-6-fluoro-spiro-(chroman-4, 4'-imidazolidine)-2', 5'-dione) and the chromones (4) 7-hydroxy-4-oxo-4H-chromen-2-carboxylic acid, and (5) its ethyl ester is summarized in Fig. 1. The rank order of inhibitory potency, expressed in terms of IC₅₀ values, was 3 > 4 > 5 > 2 > 1 in HLAR. This compared well with a potency rank of 3 > 4 > 5 > 1,2 in HPAR but not with the rank of 3 > 2 > 1 > 4,5 obtained in RLAR.

Although the overall potency ranks of these inhibitors with the human enzymes were similar, the compounds produced unpredictable differences in their ability to inhibit HLAR and HPAR compared to RLAR. However, with the exceptions of chromones 4 and 5, all compounds were generally less potent in HPAR than in RLAR and equipotent to RLAR in HLAR. The inhibitory potency of Alrestatin (compound 1) remained the same in HLAR and RLAR but decreased fourfold in HPAR. Quercitin (compound 2) was also equipotent in HLAR and RLAR compared to RLAR. The chromone 4 was equipotent in HPAR and RLAR and increased fivefold in HLAR. Its ethyl ester (compound 5) increased fourfold in HLAR but decreased twofold in HPAR compared to RLAR.

To further compare the inhibitory susceptibility of HLAR vs. HPAR and RLAR, a series of antiallergy compounds possessing aldose reductase inhibitory activity were evaluated (Fig. 2). These previously unreported compounds were chosen because of their various potency differences in HPAR vs. RLAR and because of their structural diversity. Compound 6 (2-tetrazolyl-3-chloro-4-oxo-4H-chromen) was equipotent to chromones 4 and 5 in RLAR. In HPAR this compound was 16-fold less potent compared to no differences for acid 4 and a twofold decrease for ester 5. In HLAR compound 6 displayed a 1.8-fold increase in activity compared to a 5-fold increase of the acid 4 and a 4-fold increase for the ester 5. Compound 7 (3,3'-m-phenylene-di-5-isoxazolecarboxylic acid) displayed a twofold decrease in HPAR and a twofold increase in HLAR compared to RLAR. Compound 8 (3'-carboxy-2'-hydroxy-5'-nitrooxanilic acid, ethyl ester) displayed a decrease over 70-fold in HPAR compared to RLAR and a 1.6 fold increase in HLAR. Compound 9 ([2-carboxy-1,4-dihydro-8-methyl-4-oxo-7-quinolyl] oxamic acid) displayed a 17-fold decrease in HPAR and a twofold increase in HLAR. Finally, compound 10 (10-chloro-1,4,6,9-tetrahydro-4,6-dioxopyrido[3,2-g]quinoline-2,8-dicarboxylic acid) displayed an approximate twofold decrease in HPAR and a twofold increase in HLAR. The rank order of potency for these compounds in HLAR was 8 > 9 > 10 > 6 > 7. Unlike the results in Fig. 1, this correlated well with RLAR but differed with that in HPAR (10 > 8 > 9 > 7 > 6).

<table>
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<th>Compound</th>
<th>HLAR IC₅₀ x10⁻⁶</th>
<th>HPAR IC₅₀ x10⁻⁶</th>
<th>RLAR IC₅₀ x10⁻⁶</th>
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Fig. 1. Summary of inhibitions, expressed in terms of the IC₅₀ of known aldose reductase inhibitors in HLAR, HPAR, and RLAR.
HPAR, and RLAR to be inhibited by compounds of wide structural diversity, large significant differences were observed. These differences may be due in part to different degrees of bulk tolerance exhibited by the various enzymes. In general, HPAR appeared to be less susceptible to inhibition than either HLAR or RLAR, with the susceptibility of HLAR being more similar to RLAR than to HPAR. Specific trends, however, could not be predicted. The decreased susceptibility of HPAR was not merely due to nonspecific binding of inhibitor to inert protein, since the same inhibitory profile was obtained with either the more stable ammonium sulfate-fractionated or purified enzyme preparation. Moreover, other inhibitors including chromone 4 have increased potency in HPAR (unpublished results).

Fluctuations in potencies of these inhibitors to aldose reductase from various sources indicate that no universal inhibitor exists. Of the compounds evaluated, Sorbinil (compound 3) appeared overall to be the most potent, although the oxanilic acid 9 and ester 8 were comparable against HPAR. These findings indicate that the evaluation of aldose reductase inhibitors for potential clinical use may require the use of human aldose reductase from the appropriate target tissue.

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REFERENCES