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KINETIC CHARACTERISTICS OF ZENECA ZD5522, A POTENT INHIBITOR OF HUMAN AND BOVINE LENS ALDOSE REDUCTASE

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Abstract—Aldose reductase (aldehyde reductase 2) catalyses the conversion of glucose to sorbitol, and methylglyoxal to acetol. Treatment with aldose reductase inhibitors (ARIs) is a potential approach to decrease the development of diabetic complications. The sulphonylnitromethanes are a recently discovered class of aldose reductase inhibitors, first exemplified by ICI 215918. We now describe enzyme kinetic characterization of a second sulphonylnitromethane, 3′,5′-dimethyl-4′-nitromethylsulphonyl-2-(2-tolyl)acetanilide (ZD5522), which is at least 10-fold more potent against bovine lens aldose reductase *in vitro* and which also has a greater efficacy for reduction of rat nerve sorbitol levels *in vivo* (ED_{95} = 2.8 mg kg^{-1} for ZD5522 and 20 mg kg^{-1} for ICI 215918). ZD5522 follows pure noncompetitive kinetics against bovine lens aldose reductase when either glucose or methylglyoxal is varied ($K_{is} = K_{ii}$ = 7.2 and 4.3 nM, respectively). This contrasts with ICI 215918 which is an uncompetitive inhibitor (K_{ii} = 100 nM) of bovine lens aldose reductase when glucose is varied. Against human recombinant aldose reductase, ZD5522 displays mixed noncompetitive kinetics with respect to both substrates (K_{is} = 41 nM, K_{ii} = 8 nM with glucose and K_{is} = 52 nM, K_{ii} = 3.8 nM with methylglyoxal). This is the first report of the effects of a sulphonylnitromethane on either human aldose reductase or utilization of methylglyoxal. These results are discussed with reference to a Di Iso Ordered Bi Bi mechanism for aldose reductase, where the inhibitors compete with binding of both the aldehyde substrate and alcohol product. This model may explain why aldose reductase inhibitors follow noncompetitive or uncompetitive kinetics with respect to aldehyde substrates, and X-ray crystallography paradoxically locates an ARI within the substrate binding site. Aldehyde reductase (aldehyde reductase 1) is closely related to aldose reductase. Inhibition of bovine kidney aldehyde reductase by ZD5522 follows uncompetitive kinetics with respect to glucuronate (K_{ii} = 39 nM), indicating a selectivity greater than 5-fold for bovine aldose reductase relative to aldehyde reductase.

Key words: aldehyde reductase; aldose reductase; aldose reductase inhibitor; diabetes mellitus drug therapy; diabetes mellitus enzymology; enzyme inhibitors

Aldose reductase (aldehyde reductase 2, ALR2§) (EC 1.1.1.21) catalyzes the NADPH-dependent reduction of a wide variety of aldehydes to their corresponding alcohols. The enzyme catalyzes the reduction of the open-chain form [1, 2] of glucose to sorbitol. Excessive flux through this pathway has been implicated in the damage to nerve, retina, lens and kidney which is associated with long-term diabetic complications [3]. Recently, methylglyoxal has been shown to be a good substrate for ALR2 and a model of diabetic complications has been proposed in which it plays a major role [4]. The mechanisms of tissue damage in diabetes appear to

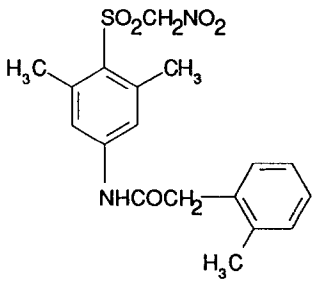

Fig. 1. Structure of ZD5522, 3′,5′-dimethyl-4′-nitromethylsulphonyl-2-(2-tolyl)acetanilide.

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§ Abbreviations: ALR1, aldehyde reductase 1, aldehyde reductase; ALR2, aldehyde reductase 2, aldose reductase; ALR2I, ALR2 which is insensitive to inhibitors; ALR2S, ALR2 which is sensitive to inhibitors; ARI, aldose reductase inhibitor; E, enzyme; E*, enzyme in modified conformation; ED_{95}, dose required to give 95% effect; I, inhibitor; IC_{50}, concentration giving 50% inhibition; ICI 215918, (2,6-dimethylphenylsulphonyl)-nitromethane; k_{cat}, turnover number; K_{is} and K_{ii}, dissociation constants for inhibitor at zero and saturating substrate concentrations respectively; S, substrate; ZD5522, 3′,5′-dimethyl-4′-nitromethylsulphonyl-2-(2-tolyl)acetanilide.

involve the following: decreased myoinositol, altered Na^+/K^+ ATPase activity, covalent modification of proteins by increased glucose and acetol (produced from methylglyoxal), accumulation of sorbitol and fructose in the cytoplasm leading to hypertonicity and osmotic uptake of water, and reduced NADPH, resulting in oxidative stress [3–5]. ARIs have been shown to delay the onset of, prevent, and in some cases, to reverse tissue damage in diabetic animals [3]. Inhibition of ALR2, therefore, offers a potential approach to the treatment of diabetic complications in man. The sulphonylnitromethanes are a recently discovered class of ARIs, with ICI 215918 being the first to be characterized in terms of enzyme kinetics [6]. ZD5522 (Fig. 1) [7] is also a sulphonylnitromethane and has been selected for detailed study because of its higher potency *in vitro* and *in vivo*. We present characterization of inhibition of human recombinant and bovine lens ALR2 by ZD5522. This work includes the first description of the effects of a sulphonylnitromethane on either human ALR2 or utilization of methylglyoxal.

A possible problem during therapy with ARIs is specificity for the target enzyme. Aldehyde reductase (aldehyde reductase 1, ALR1) (EC 1.1.1.2) exhibits high homology to ALR2 in terms of structure and function [8, 9]. We have probed the selectivity of ZD5522 by characterizing the inhibition of bovine ALR1.

Both ALR1 and ALR2 appear to follow a compulsory ordered mechanism where NADPH is the first substrate to bind and $NADP^+$ is the last product to dissociate [10–14]. Protein fluorescence and kinetic studies indicate that ALR2 undergoes conformation changes on binding and dissociation of nucleotide, showing that a Di Iso Ordered Bi Bi mechanism is followed [13, 14]. The observed kinetics of inhibition of ALR2 are discussed in terms of this mechanism. A model is proposed which may explain the apparent paradox where ARIs follow noncompetitive or uncompetitive kinetics with respect to aldehyde substrates (reviewed in Ref. 3), but X-ray crystallography locates an ARI within the substrate binding site [15].

MATERIALS AND METHODS

Materials

Reagents were purchased from the Sigma Chemical Co. (Poole, Dorset, U.K.), BDH (Lutterworth, U.K.) and Unipath Ltd (Basingstoke, Hants, U.K.). Bovine ALR2 and ALR1 were partially purified as previously described [16]. Human recombinant ALR2 was purified to homogeneity as described by Petrash *et al.* [17]. ZD5522 was synthesized at ZENECA Pharmaceuticals [7].

Methods

Measurement of ARI potency in vivo. The potency of orally administered ARIs was determined by measuring sorbitol levels in the sciatic nerves of rats with streptozoticin-induced diabetes [18].

In vitro *assay of ALR2 and ALR1.* Assays were performed at 37° in phosphate buffered saline (137 mM NaCl, 2.7 mM KCl, 8.1 mM Na_2HPO_4 and 1.5 mM KH_2PO_4), pH 7.3, containing 100 μM NADPH. With human recombinant ALR2, glucose was varied from 0 to 250 mM and methylglyoxal from 0 to 80 μM. With bovine ALR2, glucose was varied from 0 to 100 mM and methylglyoxal from 0 to 16 μM. For bovine kidney ALR1, glucuronate was varied from 0 to 2 mM. The reaction was initiated by the addition of enzyme to about 25 nM for ALR2, and protein to approximately 0.2 mg mL^{-1} for ALR1 and then followed spectrophotometrically at 340 nm, taking the extinction coefficient of NADPH as 6200 M^{-1} cm^{-1}.

Protein concentration. This was determined by the Folin method.

Kinetic analysis. All data were analysed by multivariate nonlinear regression using the program 'GraFit' [19]. The best fit equations are given in the Results section. A suitable rate equation to describe each data set was identified as follows. An *F*-test was used to compare the residual sum of squares following fitting of a number of different equations. The most suitable rate equation was chosen on the basis of having the largest number of fitting parameters, none of which was redundant [20]. The *F*-test estimates the probability, P, that the improvement in fit associated with the extra parameter is due to chance alone. The term is justified by the data if P is low (<1%) and rejected if P is high (>5%). When the most suitable equation had been identified, the quality of fit was assessed by the following criteria. The parameter values and standard errors had to be reasonable, and the residual differences between observed and calculated rates had to be small and show a random distribution [20].

RESULTS

Potency of ZD5522 in vivo

During hyperglycaemia, ALR2 activity is thought to lead to accumulation of sorbitol in nerves and consequent neuropathy. Based on studies with the ALR2 inhibitor ponalrestat, it is believed that nerve conduction can be restored in diabetic rats by a 95% reduction in rat sciatic nerve sorbitol levels [21]. The dose of compound giving this effect is termed ED_{95}. Similar work on ZD5522 (unpublished results) also indicates that a large reduction in sorbitol levels is required to restore nerve conduction in diabetic rats. ED_{95} values for ZD5522 and ICI 215918 are 2.8 and 20 mg kg^{-1}, respectively. These studies indicate that, in terms of ED_{95}, ZD5522 is seven times more potent than ICI 215918.

Inhibition of bovine lens and human recombinant ALR2 by ZD5522

Our preparations of bovine ALR2 follow biphasic kinetics with respect to both substrate and inhibitor. This behaviour has previously been reported for ALR2 from several sources [6, 14, 22]. The form with a high affinity for glucose is sensitive to inhibitors and is referred to as ALR2S. The form with a low affinity for glucose is insensitive to inhibitors and is referred to as ALR2I. Only ALR2S was characterized in detail because ALR2I activity was very low at physiological levels of glucose and was difficult to measure with accuracy.

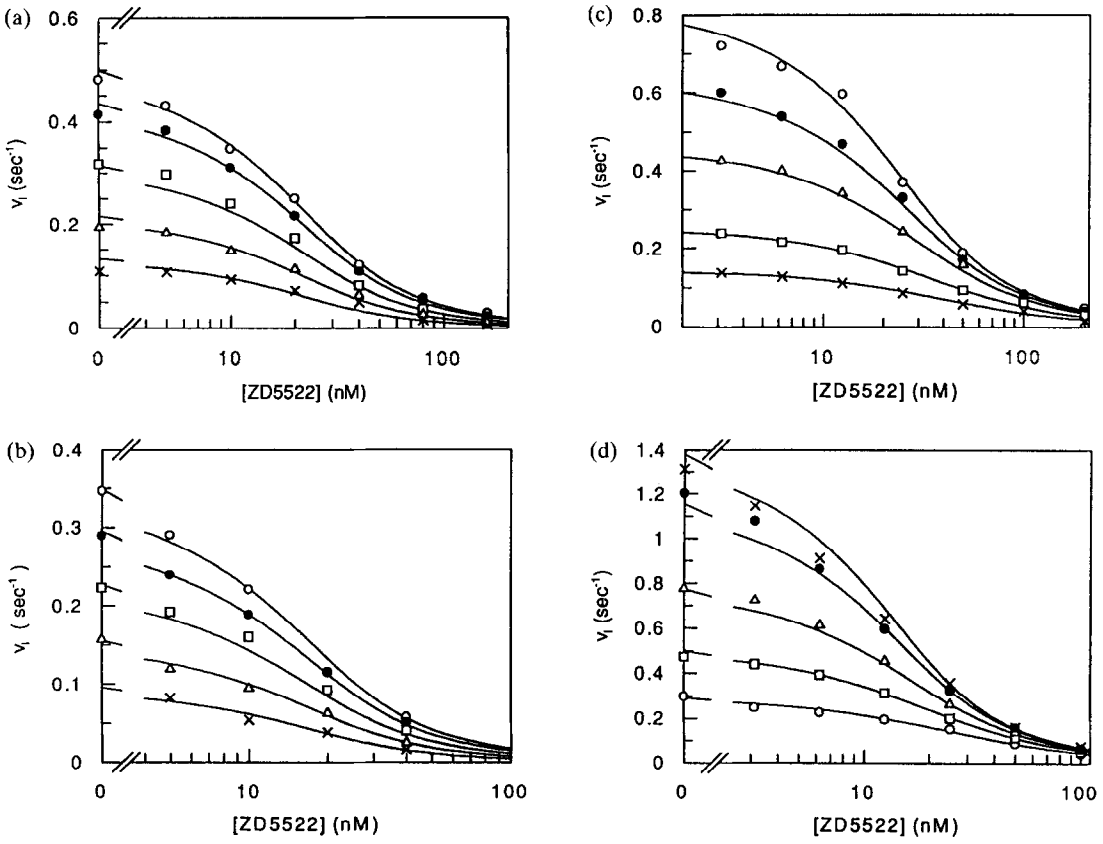

Fig. 2. Plots for inhibition of bovine lens and human recombinant ALR2 by ZD5522 with varying glucose or methylglyoxal. Assays were performed as described in Materials and Methods, and data analysis as described in Results. The best fit lines are shown as v against $[I]$ plots at different substrate concentrations and were calculated by fitting equation 1, where K_i' is allowed to vary according to equation 3. The rate is plotted as moles NADPH utilized per mole of enzyme per second (units sec^{-1}). The enzyme concentration was estimated using equation 1. In order to aid clarity, values are plotted as the means of duplicates which vary by less than 15%. ZD5522 concentration is plotted on a $\log_{10}$ scale. (a) Bovine lens ALR2 with glucose: (○) 100 mM; (●) 50 mM; (□) 25 mM; (△) 12.5 mM; (×) 6.25 mM. (b) Bovine lens ALR2 with methylglyoxal: (○) 16 μM; (●) 8 μM; (□) 4 μM; (△) 2 μM; (×) 1 μM. (c) Human recombinant ALR2 with glucose: (○) 250 mM; (●) 100 mM; (△) 50 mM; (□) 20 mM; (×) 10 mM. (d) Human recombinant ALR2 with methylglyoxal: (×) 100 μM; (●) 50 μM; (△) 20 μM; (□) 10 μM; (○) 5 μM.

The mechanism of inhibition was determined when varying either glucose or methylgloxal (Fig. 2(a)–(d) and Table 1). All data were analysed by multivariate non-linear regression (see Methods). Tight binding kinetics are observed in all cases, allowing the turnover number (number of moles of product per mole of enzyme per second, k_{cat} value) to be estimated by assuming that the concentration of active enzyme equals the calculated concentration of inhibitor binding sites. Equation 1 [22] can be fitted to the observed initial rates:

$$v_i = 0.5v_0\{-K_i/[E]_t - [I]/[E]_t + 1 + \sqrt{((K_i'/[E]_t + [I]/[E]_t - 1)^2 + 4K_i'/[E]_t)}\} + \alpha[S] \quad (1)$$

where $\alpha = V_{max}/K_m$ for ALR2I. The high K_m and K_i' values for ALR2I mean that its activity is proportional to the substrate concentration under our experimental conditions. The human recombinant enzyme does not exhibit any significant ALR2I activity so that the $\alpha[S]$ term is not required. $[E]_t$ is the concentration of inhibitor binding sites and v_0 is given by the Michaelis–Menten relationship:

$$v_0 = k_{cat}[E]_t[S]/(K_m + [S]) \quad (2)$$

K_i' is the apparent dissociation constant for the inhibitor according to equation 3 [22]:

$$K_i' = K_{is}K_{ii}(K_m + [S])/(K_{is}[S] + K_{ii}K_m) \quad (3)$$

In the context of the present work, K_{is} is the dissociation constant for the inhibitor from complexes formed prior to association of aldehyde substrate (S) and K_{ii} is the dissociation constant from complexes formed after association of aldehyde substrate.

When either glucose or methylglyoxal is varied, ZD5522 follows noncompetitive kinetics against both

Table 1. Inhibition of bovine lens and human recombinant ALR2S by ZD5522 in the presence of glucose and methylglyoxal

Enzyme	Human recombinant		Bovine lens	
	Glucose	Methylglyoxal	Glucose	Methylglyoxal
K_{is} (nM)	41 ± 6	52 ± 12	7.2 ± 0.6	4.3 ± 0.8
K_{ii} (nM)	8.0 ± 0.7	3.8 ± 0.2	7.2 ± 0.6	4.3 ± 0.8
K_m	61 ± 3 mM	24 ± 2 μM	17 ± 1 mM	3.4 ± 0.3 μM
k_{cat} (sec^{-1})	1.0 ± 0.1	1.7 ± 0.1	0.58 ± 0.03	0.42 ± 0.08
k_{cat}/K_m ($sec^{-1} M^{-1}$)	17 ± 2	(4.2 ± 0.6) × 10^4	34 ± 2	(1.2 ± 0.3) × 10^5

Assays and data analysis were performed as described in Materials and Methods. Values were obtained by fitting equations 1 and 2, where K_i' was allowed to vary according to equation 3. For bovine lens ALR2, there was no significant improvement in fit when K_i' was allowed to vary with substrate concentration, indicating that $K_{is} = K_{ii}$. The best fit values are quoted ± SE.

bovine lens ALR2S and human recombinant ALR2. For the bovine enzyme, the value of K_{is} is approximately equal to that for K_{ii} (pure noncompetitive kinetics) (Table 1). The value of K_{is} is not equal to that of K_{ii} (mixed noncompetitive kinetics) for the human recombinant enzyme. With each substrate, the value of K_{is} is greater than K_{ii} (Table 1), indicating that the inhibitor binds with greater affinity after association of the aldehyde.

Relative to bovine lens ALR2S, the human recombinant enzyme exhibits a higher K_{is} for ZD5522 by a factor of approximately 6 to 12 (Table 1). Higher K_m (around 4- to 7-fold) and k_{cat} (about 2- to 4-fold) values are also observed for the human recombinant enzyme. Enzyme from each of the two sources displays similar K_{ii} values for ZD5522. The K_m and k_{cat} values for human recombinant ALR2 using glucose or methylglyoxal are similar to those previously published for native and recombinant human enzyme [4, 17, 23].

Inhibition of bovine kidney ALR1 by ZD5522

The observed kinetics are uncompetitive with respect to glucuronate (Fig. 3) as described by equation 4:

$$v = V_{max}[S]/\{K_m + [S](1 + [I]/K_{ii})\} \quad (4)$$

where $V_{max} = 3.5 \pm 0.4$ nmol NADPH $min^{-1} mg^{-1}$ protein, $K_m = 3.1 \pm 0.2$ mM and $K_{ii} = 39 \pm 2$ nM. The value of K_m is close to those previously determined for ALR1 from ox [10] and bovine kidney [22].

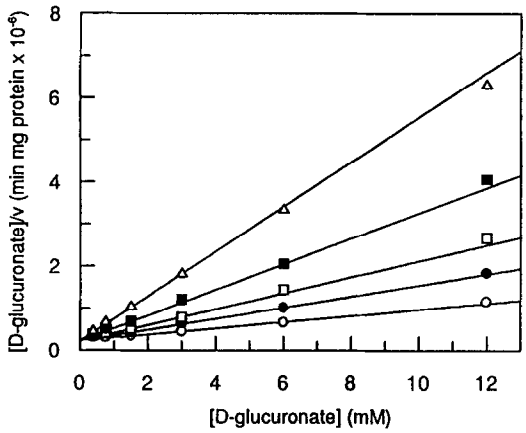

Fig. 3. Plots of inhibition of bovine kidney ALR1 by ZD5522 with varying glucuronate. Assays were performed as described in Materials and Methods, and data analysis as described in Results. The best fit lines are shown as Hanes plots at different fixed inhibitor concentrations and were calculated by fitting equation 4. In order to aid clarity, values are plotted as the means of duplicates which vary by less than 10%. (○) 0 nM; (●) 30 nM; (□) 60 nM; (■) 120 nM; (△) 240 nM.

DISCUSSION

Characteristics of ZD5522

There are numerous examples where the reported potency of an ARI varies between publications (reviewed in Ref. 24). This often seems to be due to the occurrence of two forms of the enzyme with different affinities for the inhibitor [6, 14, 22, 25]. Failure to resolve between the multiple forms leads to estimates of potency which average activity across the two phases. A change in the relative activity of the two phases then causes an apparent shift in potency. We have avoided such artefacts by successful separation of the two phases.

Detailed studies indicate a time-dependent conversion of the inhibitor sensitive form into an insensitive form [14]. For human ALR2, this appears to be reversed by thiol reagents [25]. We obtain single phase kinetics for human recombinant ALR2 which was purified in the presence of dithiothreitol. Thiol reagents do not prevent the kinetic changes in bovine ALR2 [14] and we resolved two phases for the enzyme from this source even though it was prepared in the presence of mercaptoethanol.

We present the first report of the effects of a sulphonylnitromethane on either human ALR2 or utilization of methylglyoxal. The characteristics for inhibition of utilization of methylglyoxal are very similar to those for utilization of glucose (Table 1).

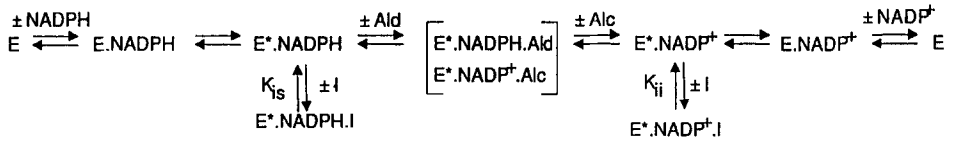

Fig. 4. Proposed kinetic mechanism for inhibition of ALR2. Note that, for some inhibitors, the K_{is} term also may reflect the binding to E·NADPH, and the K_{ii} term may be influenced by association with E·$NADP^+$ (see Discussion). E, enzyme; E*, E in modified conformation; I, inhibitor; Ald, aldehyde, Alc, alcohol.

The human and bovine enzymes have similar affinities for ZD5522 after binding of the aldehyde substrate (K_{ii}), and the human enzyme has a lower affinity prior to association with aldehyde (K_{is}). Two sulphonylnitromethanes have been characterized as inhibitors of bovine ALR2. Previous work shows that ICI 215918 follows uncompetitive kinetics when glucose is varied [6, 22], and now we demonstrate that ZD5522 exhibits noncompetitive kinetics. The converse applies for inhibition of bovine ALR1. ICI 215918 is noncompetitive and ZD5522 is uncompetitive (see Table 1 and Ref. 6). Both compounds exhibit specificity in favour of ALR2S. ZD5522 is more potent than ICI 215918 both *in vitro* and *in vivo*.

ARIs may act as analogues of aldehyde substrate and alcohol product

It has been suggested that ARIs use an allosteric site because they follow noncompetitive or uncompetitive kinetics with respect to aldehyde substrate [3]. This hypothesis conflicts with X-ray crystallography which has recently shown an ARI bound at the substrate site [15]. We now propose a model which may resolve this paradox.

Detailed kinetic studies indicate that pig muscle ALR2 follows a Di Iso Ordered Bi Bi mechanism (see Fig. 4 and Ref. 13). Binding of coenzyme induces a conformation change, E·NADPH → E*·NADPH, prior to association with the aldehyde substrate. Catalysis occurs and then the alcohol is the first product to be released prior to the rate limiting conformation change, E*·$NADP^+$ → E·$NADP^+$. Human and bovine ALR2 are likely to follow the same mechanism because there is a high degree of conservation of primary structure (86% identity in aligned amino acid sequences) across these species [8, 26]. There is also evidence for binding of coenzyme inducing conformation changes in ALR1 [10, 11] so that a Di Iso Ordered mechanism may be followed by both enzymes.

Spontaneous activation *in vitro* converts ALR2S into ALR2I (see above). This process is characterized by increases in the values of k_{cat}, K_m for aldehyde and K_{ii} for some ARIs [14]. Activation presumably involves acceleration of the rate limiting step. It has been proposed that this increase in conformational preference for E rather than E* causes reduced sensitivity to those ARIs which preferentially bind to the E* form [6]. Some ARIs have similar potency for both ALR2S and ALR2I [14]. These compounds could have similar affinities for both conformations.

ARIs seem to follow noncompetitive kinetics because they compete with both the aldehyde substrate and the alcohol product (see Fig. 4 and Ref. 6). The uncompetitive component of inhibition seems to occur as a result of the rate-limiting nature of E*·$NADP^+$ → E·$NADP^+$, which enables formation of steady state levels of E*·$NADP^+$·I [6]. The difference between uncompetitive and noncompetitive kinetics in such a model is subtle. Uncompetitive inhibitors do not bind detectably prior to association of aldehyde substrate. This could be due to differences in molecular structure, or because of small changes in the equilibrium between E·NADPH and E*·NADPH. This hypothesis is consistent with X-ray crystallography which shows the acetic acid ARI, zopolrestat, located in the substrate/product binding site [15]. There is kinetic competition between representatives of three structural types of ARIs: the acetic acids, the sulphonylnitromethanes, and the spirohydantoins [22], suggesting that they use overlapping sites. Thus, ZD5522 appears to interact with the active site of ALR2. Further evidence for this mechanism comes from the following observations.

(1) The values for K_{is} and K_{ii} are unchanged (within the limits of experimental uncertainty) when a different aldehyde substrate is used (Table 1), consistent with the idea that ARIs do not bind to an enzyme form that includes aldehyde substrate or alcohol product.

(2) For human plancental ALR2, a spirohydantoin, sorbinil, follows competitive kinetics with respect to benzyl alcohol in the reverse reaction and one of the pK values for $1/K_{is}$ is similar to that for V_{max}/K_m, suggesting that the inhibitor and alcohol use overlapping sites [27]. Two factors may explain why ZD5522 appears to act as an analogue of the aldehyde substrate and alcohol product in the forward reaction, whereas sorbinil does not function as an aldehyde analogue in the reverse reaction. First, the inhibitors have different structures, and second action as an aldehyde analogue is difficult to detect in the reverse reaction. This is because the inhibitor would have to associate with E*·NADPH or E·NADPH. The levels of these complexes are probably low in the reverse reaction because isomerization of E*·NADPH to E·NADPH is not rate-limiting for porcine ALR2 [13]. Indeed, the value of K_{is} for ZD5522 is 5–14 times higher than that of K_{ii} for human ALR2,

suggesting weaker action as an aldehyde analogue (Table 1). The other sulphonylnitromethane, ICI 215918, follows uncompetitive kinetics, suggesting that it, like sorbinil, only acts as an analogue of the alcohol product [6].

(3) $NADP^+$ protects a closely related enzyme, human liver ALR1, from covalent modification at Arg residues. Addition of gluconate (product), trimethyl glutarate or diphenylhydantoin (inhibitors) together with $NADP^+$ increases this protection [28, 29].

(4) ARIs contain groups which resemble the carbonyl moiety of aldehyde reductase substrates. This may explain the function of key groups such as the acetic acid moiety of ponalrestat, the hydantoin of sorbinil and the nitro group of ICI 215918 and ZD5522.

Apart from the X-ray crystallography studies on zopolrestat, all of this evidence on the nature of the ARI binding site is indirect. It remains possible that K_{is} and K_{ii} for ZD5522 reflect association of the inhibitor at two different sites. This hypothesis is unlikely to be correct in the light of the indirect evidence and the greater simplicity of the one site model.

Comparison of bovine lens and human recombinant ALR2

Relative to bovine ALR2S, the human recombinant enzyme has higher values for K_m(glucose), K_m(methylglyoxal) and K_{is}(ZD5522) (Table 1). These observations may reflect a higher ratio of E*·nucleotide : E·nucleotide for bovine lens ALR2S. The observation of a lower k_{cat} value for bovine lens ALR2S is also consistent with this idea because k_{cat} is primarily dependent on the rate limiting step $E^*{\cdot}NADP^+ \rightarrow E{\cdot}NADP^+$. No comparable changes in K_{ii} values are detected (Table 1), presumably because changes in conformational preference are too small to be detected by changes in this parameter. This correlation between K_m, K_{ii} and k_{cat} is predicted by the proposed mechanism of inhibition and is also consistent with the hypothesis that conversion of ALR2S to ALR2I occurs by acceleration of the rate-determining step [6].

Kinetic properties of ALR2 in the absence of inhibitors

The value k_{cat}/K_m is a measure of enzyme specificity and is the pseudo-second order rate constant when the substrate concentration is well below the K_m value [30]. The maximum value of k_{cat}/K_m is limited to about $10^9\ sec^{-1}\ M^{-1}$ by the rate of diffusion in water which controls the frequency of collisions between enzyme and substrate. For many enzyme reactions, k_{cat}/K_m falls in the range 10^6–$10^8\ sec^{-1}\ M^{-1}$. Utilization of methylglyoxal by ALR2, therefore, is close to this typical behaviour (Table 1). Glucose may appear to be a poor substrate, but ALR2 uses only the open-chain form which is around 0.003% of the total in solution [1, 2]. The value of k_{cat}/K_m for the aldehyde form, therefore, is approximately $10^6\ sec^{-1}\ M^{-1}$ and so is within the expected range.

Possible significance of the kinetics for inhibition by ZD5522

Inhibition of bovine lens ALR2S by ZD5522 follows pure noncompetitive kinetics so that the IC_{50} is independent of the concentration of glucose or methylglyoxal (Table 1). Conversely, inhibition of bovine kidney ALR1 follows uncompetititive kinetics so that the binding affinity is at a maximum when glucuronate is saturating, and decreases at lower levels of the substrate with binding being undetectable on extrapolation to zero glucuronate. Thus, selectivity for ALR2S over ALR1 is at least 5- to 9-fold, tending towards higher specificity for ALR2S as the concentration of glucuronate is reduced below saturation. However, several factors change on moving *in vivo* (e.g. ion concentrations, redox potential), with additional factors being involved (especially the presence of products and metabolism of the inhibitor). Thus, the significance of the measured selectivity for efficacy and side effects is not clear.

ALR2 catalysed utilization of both glucose and methylglyoxal may be involved in the generation of diabetic complications [4, 22]. ZD5522 is a potent inhibitor of both processes. The mixed non-competitive kinetics for inhibition of human recombinant ALR2 indicate that, as levels of glucose and methylglyoxal rise (perhaps as a result of inhibition), the concentration of ZD5522 required for 50% inhibition actually falls.

In summary, ZD5522 is a potent inhibitor of the utilization of glucose and methylglyoxal by human recombinant and bovine lens ALR2. The kinetics of inhibition are such that elevation in substrate levels will not increase the IC_{50}. ZD5522 displays selectivity for bovine ALR2S rather than ALR1. The compound also exhibits high efficacy in the reduction of sorbitol levels in rat nerve and so displays several properties suggesting that it may provide an effective treatment to reduce the development of diabetic complications.

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