Does Inhibition of Aldose Reductase Contribute to the Anti-Inflammatory Action of Setipiprant ?

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Short title

Aldose reductase inhibition by setipiprant

Key words

Setipiprant; aldose reductase inhibition; inflammation; androgenic alopecia;

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Summary

The aim of this study was to investigate aldose reductase inhibitory action of setipiprant as a potential additional mechanism contributing to its anti-inflammatory action. Aldose reductase activity was determined by spectrophotometric measuring of NADPH consumption. Setipiprant was found to inhibit aldose reductase/NADPH-mediated reduction of 4-hydroxynonenal, 4-hydroxynonenal glutathione and prostaglandin H2 substrates, all relevant to the process of inflammation. Molecular modeling simulations into the aldose reductase inhibitor binding site revealed an interaction pattern of setipiprant. Considering multifactorial etiology of inflammatory pathologies, it is suggested that, in addition to the antagonizing prostaglandin D2 receptor, inhibition of aldose reductase may contribute to the reported anti-inflammatory action of setipiprant.

Key words

Setipiprant; Asthma; Aldose reductase inhibition; Inflammation; Androgenic alopecia;

Introduction

Setipiprant, 2-[8-fluoro-2-(naphthalene-1-carbonyl)-3,4-dihydro-1H-pyrido[4,3-b]indol-5-yl] acetic acid, was initially invented as a promising remedy for allergies and inflammatory disorders based on its selective antagonism of the CRTH2 (DP2) receptor (Fretz et al. 2013; Diamant et al. 2014). As a CRTH2 antagonist, setipiprant may counteract proinflammatory effects of prostaglandin D2 (PGD2) in allergic responses. Yet inadequate efficacy revealed in clinical studies led to termination of the development of setipiprant (Norman 2014). However, the discovery that the levels of both prostaglandin D2 synthase (PTGDS) and its product PGD2 are increased in the scalp of men suffering from androgenetic alopecia (Garza et al. 2012) initiated the development of this drug as a novel treatment for baldness (Cotsarelis et al. 2015).

Setipiprant is a derivative of indole-1-acetic acid. Since compounds of this group are efficient inhibitors of aldose reductase (Van Zandt et al. 2005, 2009; Juskova et al. 2011) we were prompted to investigate the aldose reductase inhibitory activity of setipiprant. Considering the PgF2α synthase activity of aldose reductase isolated from the human placenta and mouse kidney (Kabututu et al. 2009), and more importantly the recently reported PGD2 synthase activity of recombinant human aldose reductase (AKR1B1) and mouse aldo-keto reductase AKR1B3 (Nagata et al. 2011), the aldose reductase inhibitory activity would be of great relevance for potential molecular mechanisms involved in biological action of setipiprant. The issue is also interesting with respect to potential side effects of the drug.

In the light of the above mentioned findings, we considered it interesting to study the aldose reductase inhibitory action of setipiprant in greater detail, and that at the level of isolated rat and human enzymes.

Material and methods

Chemicals

Setipiprant (Cat# Amb22015120) was obtained from Ambinter c/o Greenpharma 3, Orléans, France. Recombinant human aldose reductase AKR1B1 was kindly provided by Prof. G. Klebe, Philipps-University Marburg. The protein was expressed and purified using the protocol reported previously (Stefek et al., 2015). D,L-glyceraldehyde, sodium glucuronate, NADPH, D-glucose, β-mercaptoethanol , 4-hydroxynonenal-dimethylacetal were obtained from Sigma–Aldrich (St. Louis, MO, USA). 4-Hydroxynonenal glutathione (trifluoroacetate salt), prostaglandin H2   from Cayman Pharma , Neratovice , Czech Republic. Diethylaminoethyl cellulose DEAE DE 52 was from Whatman International Ltd. (Maidstone, England).

Animals

Male Wistar rats, 8 – 9 weeks old, weighing 200 – 250 g, were used. The animals came from the Breeding Facility of the Institute of Experimental Pharmacology, Dobra Voda (Slovak Republic). The study was approved by the Ethics Committee of the Institute and performed in accordance with the Principles of Laboratory Animal Care (NIH publication 83-25, revised 1985) and the Slovak law regulating animal experiments (Decree 289, Part 139, July 9th 2003).

Enzyme assays

Aldose reductase preparation from rat lens and aldehyde reductase purification procedure from rat kidney were reported previously (Stefek et al., 2008). Aldose reductase and aldehyde reductase activities were assayed spectrophotometrically by determining NADPH

consumption at 340 nm and were expressed as decrease of the optical density (O.D.)/s/mg protein as described previously (Stefek et al., 2008). The effect of setipiprant on the enzyme activities was determined by including in the reaction mixture the inhibitor at required concentrations dissolved in DMSO at 1% final concentration. At the same concentration, the inhibitor was included in the reference blank. The reference blank contained all the above reagents except the substrate to correct for oxidation of NADPH not associated with reduction of the substrate. The enzyme reaction was initiated by addition of a substrate and was monitored for up to 8 min after an initial period of 1 min. The substrate concentration was set to be minimally 3 times higher than the corresponding Km. Enzyme activities were adjusted by diluting the enzyme preparations with distilled water so that 0.05 ml of the preparation gave an average reaction rate for the control sample of 0.020 ± 0.005 absorbance units/min. IC_{50} values (the concentration of the inhibitor required to produce 50 % inhibition of the enzyme reaction) were determined both from the least-square analysis of the linear portion of the semi-logarithmic inhibition curves and non-linear regression analysis. Each curve was generated using at least four concentrations of inhibitor causing an inhibition in the range from at least 25 to 75 %.

Computational methods

Input conformation of setipiprant was obtained by equilibrium conformer systematic search (MMFF94) performed in the program Spartan`08 (Wavefunction Inc., USA; Shao *et al*., 2006). The pdb structure with 1z3n code (protein with $NADP^+$ and lidorestat) was taken as a model of human recombinant AKR1B1 to study the enzyme-ligand interactions. To correct the bonds and hydrogens, the enzyme structure was treated by means of the software Yasara (Krieger *et al*., 2002). The protonation state corresponding to the experimental pH value was used. First, setipiprant was immersed into unoptimized complex instead of the

lidorestat and docking was performed according to the local docking protocol of Yasara (with 250 runs and $RMSD_{min}$ = 5.0 Å). The obtained clusters were then searched for the minimum of the binding energy E_{bin} within the optimization protocol em_run.mcr, which allows optimizing the whole complex by a combination of gradient optimization, simulated annealing and single molecular dynamics calculation. The structure of a ternary complex with the minimum E_{bin} value was chosen for the interaction analysis.

Results

Inhibition of aldo-keto reductases

As shown in Table 1, setipiprant efficiently inhibited both rat and human recombinant aldose reductase. With glyceraldehyde used as a substrate, the IC_{50} for inhibition of rat aldose reductase was as low as 141 nM; about four times higher efficacy was recorded for setipiprant inhibition of human recombinant AKR1B1. Reduction of HNE, the toxic product of lipid peroxidation, and its physiological glutathione adduct by rat aldose reductase was still very efficiently inhibited by setipiprant, with the respective IC_{50} values in high nanomolar and low micromolar region.

To test the selectivity of setipiprant inhibitory action, inhibition of rat kidney aldehyde reductase was examined. By using glucuronate as a substrate, the inhibition efficacy of setipiprant was characterized by IC_{50} = 43.80 ± 0.08 µM (Table 1), giving the selectivity factor of approx. 310 in relation to rat lens aldose reductase.

In the next step, the enzyme kinetics of the inhibitory action of setipiprant was analyzed. As shown in Fig. 2, uncompetitive inhibition was observed for rat aldose reductase and glyceraldehyde as a substrate with a corresponding inhibition constant Ki = 0.065 ± 0.020 µM.

Molecular modeling

The lidorestat-bound conformation of ALR2 (PDB code: 1z3n; van Zandt et al., 2005) was used for docking owing to structural similarity of setipiprant to the ligand lidorestat. The interaction analysis of setipiprant - human recombinant AKR1B1 complex revealed efficient triple hydrogen bond connections of setipiprant with Tyr 48, His 110 and Trp 111 (Fig. 3). Six hydrophobic bonds (Trp 20, Lys 21, Phe 122, Cys 298 and Leu 300) plus one with $NADP^+$ created a hydrophobic network stabilizing the position of setipiprant in the binding site supported also by two π-π interactions (Tyr 48 and Trp 119). Moreover, the parallel interactions of setipiprant with Phe 122 and Leu 300 opened the specificity pocket.

Discussion

The double-reciprocal plot comprising parallel lines with apparently decreasing Vmax and Km values with increasing setipiprant concentrations indicates that this drug inhibits aldose reductase in an uncompetitive manner. This means that setipiprant may exert its inhibitory effect by binding to the E-$NADP^+$complex in the way the other negatively charged aldose reductase inhibitors do (Bohren and Grimshaw, 2000). Uncompetitive type of inhibition was proved for another indole-1-acetic acid derivative, sulindac (Zheng et al. 2012).

Molecular docking simulations into the aldose reductase inhibitor binding site revealed an interaction pattern of setipiprant with the enzyme which resembled that of structurally related lidorestat. On binding of these inhibitors to the aldose reductase binding site, a conformational change occurs, opening a pocket localized between Trp111 and Leu300 (Urzhumtsev et al. 1997). Since the residues lining this pocket are not conserved in aldehyde reductase, the interactions are specific for aldose reductase (El-Kabbanii et al., 1998; Sotriffer et al., 2004)

resulting in high selectivity. For setipiprant, the selectivity is characterized by a factor exceeding the value of 300.

The inflammation is a complex process of a multifactorial etiology which may be initiated by a variety of primary events. Recent studies revealed the role of reactive oxygen species and lipid peroxides in the etiology of asthma (Paredi et al., 2000; Rahman et al. 2002; Wood et al 2003; Yadav and Saini, 2016). Increased levels of hydroperoxides have been reported during senescence (Lippman, 1985, Spiteller, 2007, Trueb, 2009, Yavuzer et al., 2016).

Lipid peroxidation products, namely toxic aldehydes, are considered important mediators of the inflammation process initiated by reactive oxygen species. Aldose reductase was found to reduce peroxidation derived lipid aldehydes, such as 4-hydroxy-trans-2-nonenal (HNE) and their glutathione conjugates (e.g. GS-HNE), to corresponding alcohols, 1,4-dihydroxy-nonene (DHN) and glutathionyl-1,4-dihydroxynonene (GS-DHN), respectively, which take part in the inflammation signaling . The reduced aldehyde glutathione conjugate GS-DHN is considered a novel signaling intermediate in the transduction of reactive oxygen species initiated cell signals, leading eventually to inflammation response (Srivastva et al. 2011).

As a matter of fact, the key step of the inflammation cascade initiated by reactive oxygen species/lipid hydroperoxides is dependent on aldose reductase, an essential component of the physiological detoxification system. Inhibition of aldose reductase was found to prevent significantly the transfer of the inflammatory signals induced by multiple factors including allergens, cytokines, growth factors, endotoxins, high glucose, and autoimmune reactions at the cellular level as well as in animal models (Srivastava et al. 2011; Ramana and Srivastava, 2010). Aldose reductase inhibitors thus present a promising therapeutic approach to deal with a number of inflammatory diseases such as asthma (Ramana et al., 2011, Yadav et al., 2009, 2011, 2013), uveitis (Yadav et al., 2007, 2010, Di Fillipo 2014), sepsis (Reddy et al. 2009;

Pandey 2012), periodontitis (Kador et al. 2011) and other injuries that have the potential of stimulating the immune system and generating large amounts of inflammatory cytokines and chemokines. The ability of setipiprant to inhibit efficiently aldose reductase-mediated reduction of HNE and its physiological adduct GS-HNE thus represents a plausible mechanism which may contribute to the anti-inflammatory action of setipiprant.

Recently, Nagata et al. (2011) reported catalytic activity of AKR1B1 in isomerization of PGH2 to PGD2 in the absence of NADPH. In the view of our findings revealing the inhibitory action of setipiprant on NADPH-dependent reduction of PGH2 by AKR1B1, we hypothesize that setipiprant may affect the production of PGD2 via NADPH-independent isomerization of PGH2 as well. Of course, this hypothesis needs experimental proof which was beyond the scope of this report.

Conclusions

Aldose reductase inhibitory action of setipiprant was proved and molecular docking simulations into the aldose reductase inhibitor binding site revealed an interaction pattern of setipiprant with the enzyme. Considering multifactorial etiology of inflammatory disorders, it is suggested that, in addition to the antagonizing prostaglandin D2 receptor, inhibition of aldose reductase may contribute to the reported anti-inflammatory action of setipiprant.

Conflict of interest

The authors declare that they have no conflicts of interest to disclose.

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Table 1. Inhibition of aldo-keto reductases by setipiprant

Enzyme	Substrate (mM)		Km (mM)	IC$_{50}$ (μM)[a]
	Glyceraldehyde	4.67	0.585[b]	0.141 ± 0.053
Rat lens ALR2	GS-HNE	0.10	0.030[c]	1.190 ± 0.194
	HNE	0.50	0.030[d]	0.571 ± 0.064
Rat kidney ALR1	Glucuronate	20.00	2.240[e]	43.800 ± 0.080
Human recombinant AKR1B1	Glyceraldehyde	4.67	0.092[f] 0.124[g]	0.038 ± 0.004
	PGH2	0.02	0.002[h]	2.076 ± 0.103

HNE, 4-hydroxynonenal; GS-HNE, 4-hydroxynonenal glutathione; PGH2, prostaglandin H2; [a]Results are mean values ± SD from at least three measurements. [b]Stefek et al., 2008; [c]Srivastava et al., 1998; [d]Endo et al., 2009; [e]Unpublished results [f]Balendiran et al., 2005; [g]Kaiserova et al., 2006, [h]Kabututu et al., 2009.

Figure legends

Figure 1. Chemical structure of setipiprant.

Figure 2. Inhibitory effect of setipiprant on rat lens aldose reductase. Typical double reciprocal plots of the initial enzyme velocity vs. concentration of substrate (D,L-glyceraldehyde) in the presence or absence of setipiprant: (■) no inhibitor; (○) 0.1 μM; (●) 0.2 μM setipiprant (uncompetitive type of inhibition, $K_i = 0.065 \pm 0.020$ μM).

Figure 3. Binding mode of setipiprant (dark blue) with human aldose reductase (AKR1B1 - pdb model 1z3n; cyan) and $NADP^+$ (magenta). The oxygen atoms of the carboxylate group of the inhibitor form H-bonds to Tyr 48, His 110 and Trp 111 (yellow dashed lines). The position of setipiprant is stabilized by hydrophobic (green) and π-π (red) interactions.
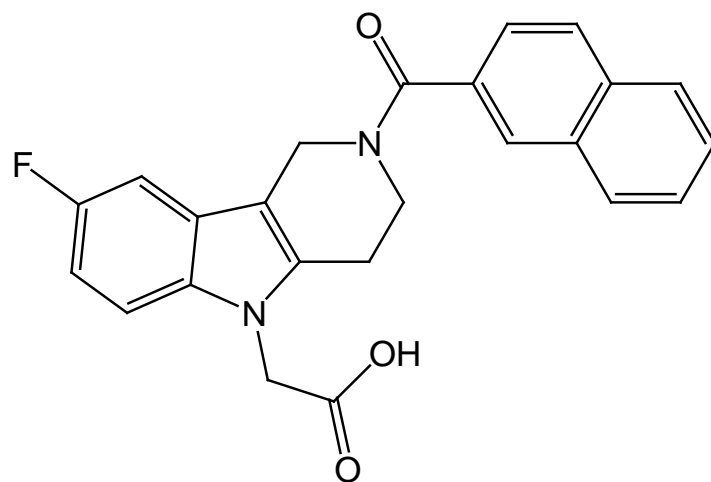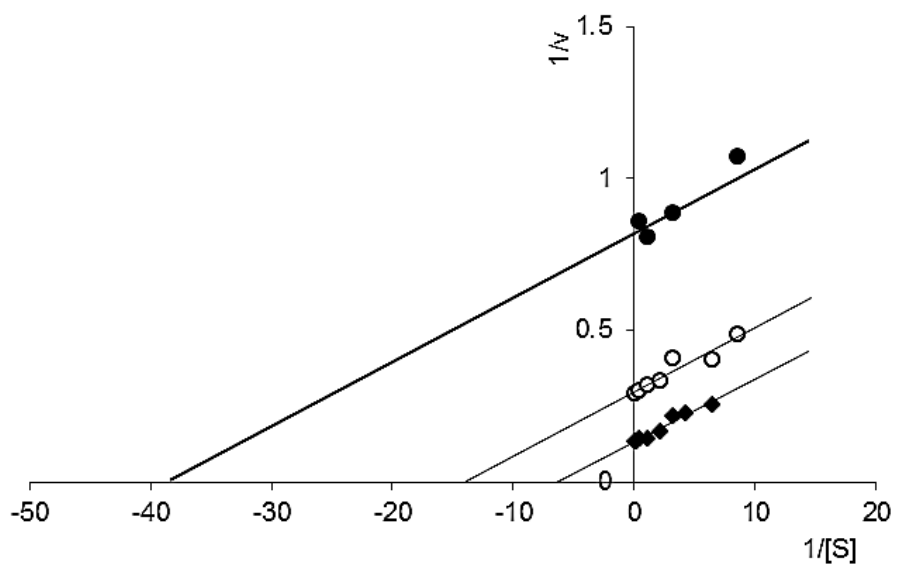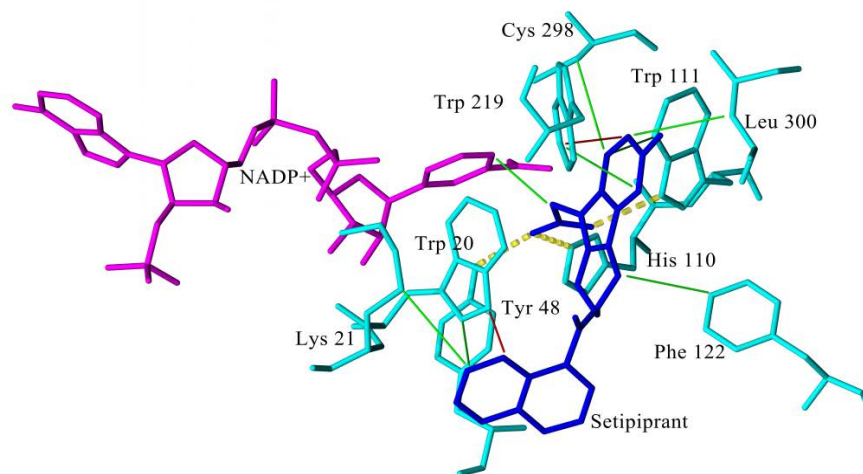
Fig. 1

Fig. 2

Cys 298
Trp 111
Trp 219
Leu 300
NADP+
Trp 20
His 110
Tyr 48
Lys 21
Phe 122
Setipiprant

Fig. 3