Available online at www.sciencedirect.com
ScienceDirect
European Journal of Pharmacology 584 (2008) 213–221
www.elsevier.com/locate/ejphar

Inhibiting wild-type and C299S mutant AKR1B10; a homologue of aldose reductase upregulated in cancers

Malkhey Verma [a,1], Hans-Joerg Martin [b,1], Wahajul Haq [c], Timothy R. O'Connor [d], Edmund Maser [b], Ganesaratnam K. Balendiran [a,*]

[a] Division of Immunology, Beckman Research Institute, City of Hope National Medical Center, 1450 E. Duarte Road, Duarte, CA 91010, USA
[b] Institute of Toxicology and Pharmacology for Natural Scientists, University Medical School Schleswig-Holstein, Campus Kiel, Brunswiker Str. 10, 24105 Kiel, Germany
[c] Medicinal Chemistry Division, Central Drug Research Institute, Lucknow 226001, India
[d] Division of Biology, Beckman Research Institute, City of Hope National Medical Center, Duarte, California 91010, USA

Received 11 June 2007; received in revised form 5 December 2007; accepted 24 January 2008
Available online 5 February 2008

Abstract

AKR1B10 is an aldose reductase (AR) homologue overexpressed in liver cancer and various forms of that enzyme in carcinomas catalyze the reduction of anticancer drugs, potential cytostatic drug, and DL-glyceraldehyde but do not catalyze the reduction of glucose. Kinetic parameters for wild-type and C299S mutant AKR1B10 indicate that substitution of serine for cysteine at position 299 reduces the affinity of this protein for DL-glyceraldehyde and enhances its catalytic activity. Fibrates suppress peroxisome proliferation and the development of liver cancer in human. Here we report the potency of fibrate-mediated inhibition of the carbonyl reduction catalyzed by wild-type and C299S mutant AKR1B10 and compare it with known AR inhibitors. Wild-type AKR1B10-catalyzed carbonyl reduction follows pure non-competitive inhibition kinetics using zopolrestat, EBPC or sorbinil, whereas fenofibrate, Wy 14,643, ciprofibrate and fenofibric acid follow mixed non-competitive inhibition kinetics. In contrast, catalysis of reaction by the C299S AKR1B10 mutant is not inhibited by sorbinil and EBPC. Despite these differences, the C299S AKR1B10 mutant still manifests kinetics similar to the wild-type protein with other fibrates including zopolrestat, fenofibrate, Wy 14,346, gemfibrozil and ciprofibrate that show mixed non-competitive inhibition kinetics. The reaction of the mutant AKR1B10 is inhibited by fenofibric acid, but manifests pure non-competitive inhibition kinetics that are different from those demonstrated for the wild-type enzyme.

Keywords: AKR1B10; Fenofibrate; Cancer; Liver; Lung

1. Introduction

Aldo–keto reductase (AKR) protein family member, AKR1B10, is overexpressed in most cases of squamous cell carcinoma (SCC) and adenocarcinoma both of which are associated with smoking (Fukumoto et al., 2005). AKR1B10 belongs to AR subfamily (AKR1B) and was discovered as an enzyme overexpressed in human liver cancers (Cao et al., 1998; Hyndman and Flynn, 1998; Jez et al., 1997; Scuric et al., 1998). Furthermore, the anticancer drugs daunorubicin, which is used in the treatment of lung cancer, and oracin, a potential cytostatic drug for oral use, which is, at present, in phase II of clinical trials, are reduced by AKR1B10 (Martin et al., 2006). The carbonyl groups in the daunorubicin and oracin are converted to the corresponding alcohols and inactivated by AKR1B10. The question arises concerning how drugs containing a carbonyl moiety can be used for successful chemotherapy of liver cancers in the presence of overexpressed AKR1B10? To address this issue we explored AR inhibitors, which may provide insight for the development of combination drugs to solve that problem.

Using a novel approach similar to fragment-based, structure-guided inhibitor design we have demonstrated that AR is a target of action for several fibrate derivatives (Balendiran and Rajkumar,

* Corresponding author. Fax: +1 626 301 8186.
E-mail address: gbalendiran@coh.org (G.K. Balendiran).
[1] These authors contributed equally to this study.

0014-2999/$ - see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2008.01.036

2005; Balendiran et al., 2007; Klemin et al., 2006). Fibrates, which are also known as peroxisome proliferators, lead to the development of liver tumors in rats and mice (Hess et al., 1965; Reddy et al., 1980). However, humans appear to be resistant to the induction of peroxisome proliferation and the development of liver cancer by fibrates (Gariot et al., 1983; Gonzalez, 2002). Understanding the role of AKR1B10 in fibrate action is critical since: 1) AKR1B10 is a member of AKR family which shows a high sequence homology with AR. 2) AKR1B10 and AR like molecules are overexpressed in liver and lung cancers as well as hepatocellular and squamous cell carcinomas.

Residue Cys299 in AKR1B10, equivalent to the Cys298 found in the active site vicinity of AR, is conserved in many members of aldo–keto reductase family of proteins. Several lines of evidence distinguish Cys298 as an important regulatory site on AR. In AR, conversion of Cys298 to serine (C298S) resulted in an enzyme form that is resistant to modification with reagents that are known to cause functional changes in enzyme activity (Bohren and Gabbay, 1993; Petrash et al., 1992). Residue Cys298 of AR is also a site for thiolation by oxidized glutathione (Cappiello et al., 1996) and glutathiolated AR is catalytically inactive (Cappiello et al., 1996). The carbonyl containing compound, 4-hydroxy-2-nonenal (HNE) modifies AR predominantly at the Cys298 position, resulting in an enzyme form with reduced sensitivity to AR inhibitors. Therefore, the residue Cys299 in AKR1B10 may play a sensitive role in the inhibition properties of compounds.

Several AR inhibitors, for example sorbinil, have reached human clinical trials, but have been withdrawn due to adverse side effects (Group, 1990; Vander Jagt et al., 1996). The adverse side effects are suspected to occur via a closely-related enzyme of the AKR family, aldehyde reductase (AKR1A1, EC 1.1.1.2) (Sato and Kador, 1990; Vander Jagt et al., 1996). Aldehyde reductase and AR are functionally linked through broad and overlapping substrate specificity (Bohren et al., 1989). Most importantly many AR inhibitors inhibit aldehyde reductase, consequently impeding proper AR. The striking difference between AR and aldehyde reductase is Cys298. Since Cys298 is not conserved in aldehyde reductase, understanding the contribution of the related Cys299 residue in AK1B10 will not only offer an indirect approach to study the side effects caused by aldehyde reductase against the potential inhibitors but will also help evaluate the regulatory role of this residue. Therefore, understanding the contribution of the Cys299 residue is essential in the rational design of a compound that specifically regulates AKR1B10.

In the present study we have characterized the potential of several fibrate derivatives in the inhibition of carbonyl reduction activity of purified recombinant human wild-type and the C299S mutant form of AKR1B10 using DL-glyceraldehyde as the substrate. We have also tested the effectiveness of these compounds to inhibit the AKR1B10-catalyzed reduction of the anticancer drug daunorubicin.

2. Materials and methods

All the reagents used in the study were obtained from Sigma-Aldrich Chemical Company (St. Louis, USA) with the exception of EBPC, which was purchased from Tocris, USA, and sorbinil and zopolrestat were gift from Pfizer. Fenofibric acid was prepared and characterized following the procedures we reported earlier (Rath et al., 2005).

2.1. Expression and purification of recombinant wild-type and C299S mutant AKR1B10

AKR1B10 gene in pQE-70 (QIAGEN) vector was transformed in SG13009 cells (QIAGEN, Valencia, USA). The cells were grown in Luria–Bertani broth containing ampicillin (100 μg/ml) and kanamycin (25 μg/ml) with constant shaking to reach the OD_{600} = 0.6–0.8 at 37 °C at 240 rpm. The protein expression was induced by supplementing 1 mM isopropyl-1-thio-galactopyranoside (IPTG) in the culture medium. The cells were harvested after 5 h by centrifugation (6000 *g*, 10 min) and resuspended in 20 mM Tris–HCl buffer (pH 7.4) containing 100 mM NaCl, 10 mM imidazole and 1 mM 2-mercaptoethanol and lysed by sonication. Wild-type AKR1B10 was isolated from the lysate separated by centrifugation at 10,000 *g* for 45 min at 4 °C. The supernatant containing hexa-His-AKR1B10 was incubated for 1–2 h by constant gentle mixing with Talon metal affinity matrix (Clontech, Mountain View, USA), later matrix slurry was passed through column and washed with 20 mM Tris buffer (pH 7.4) having 100 mM NaCl and 1.0 mM 2-mercaptoethanol. The protein was eluted with 150 mM imidazole in 20 mM Tris buffer (pH 7.4) containing 100 mM NaCl and 1 mM 2-mercaptoethanol and dialyzed in the 20 mM Tris buffer (pH 7.4) containing 1 mM 2-mercaptoethanol. His-AKR1B10 wild-type protein was further purified by anion exchange on DEAE Sephadex A25 column by negative binding with DEAE Sephadex A25 matrix. The concentration of the AKR1B10 proteins was determined by the Bradford assay (Bio-Rad, Hercules, USA) (Bradford, 1976), the purity was assessed by SDS-PAGE (Fig. 1) and the enzyme activity was determined by using 10 mM DL-glyceraldehyde and 0.2 mM NADPH as substrate and cofactor, respectively. Recombinant C299S mutant AKR1B10 was overexpressed and purified (Fig. 1) following the procedures described above for the wild-type AKR1B10.

2.2. Kinetics of DL-glyceraldehyde reduction by AKR1B10

The carbonyl reduction activity of his-tagged AKR1B10 wild-type and C299S mutant proteins were monitored spectrophotometrically, by measuring decrease in the absorbance of the cofactor NADPH at 340 nm (Balendiran and Rajkumar, 2005; Crosas et al., 2003; Nishimura et al., 1991) and 25 °C with time course for 3 min. The assay was carried out in 100 mM sodium phosphate buffer (pH 7.5) using 0.2 mM NADPH, 0.3 μM (wild-type and C299S mutant) AKR1B10, and varied concentrations (0.5–7.5 mM for wild-type and 1.0–100 mM for C299S) of DL-glyceraldehyde and inhibitors (zopolrestat, fenofibrate, Wy 14,643, sorbinil, ciprofibrate, fenofibric acid, gemfibrozil, EBPC (Fig. 2)). One unit of AKR1B10 enzyme activity is defined as μmoles of NADPH oxidized/min.