CORE
Metadata, citation and similar papers at core.ac.uk
Provided by Archivio della Ricerca - Università di Pisa

Kinetic features of carbonyl reductase 1 acting on glutathionylated aldehydes

Roberta Moschini[1,a], Rossella Rotondo[1,a], Giovanni Renzone[2], Francesco Balestri[1], Mario Cappiello[1], Andrea Scaloni[2], Umberto Mura[1] and Antonella Del-Corso[1*]

[1]: University of Pisa, Department of Biology, Biochemistry Unit, via S. Zeno, 51, Pisa (Italy)

[2]: Proteomics & Mass Spectrometry Laboratory, ISPAAM-CNR, Via Argine, 1085, Napoli (Italy)

[a]: These authors contributed equally to the work

*Corresponding author: Antonella Del-Corso, University of Pisa, Department of Biology, Biochemistry Unit, via S. Zeno, 51, 56123, Pisa, Italy.

Phone: +39.050.2211454; Fax: +39.050.2211460
E-mail: antonella.delcorso@unipi.it

Abstract

The attempt to evaluate the human carbonyl reductase 1 (CBR1) activity on 3-glutathionylated-4-hydroxyalkanals through the classical spectrophotometric assay in which NADPH oxidation is monitored at 340 nm failed. This was due to the ability of the enzyme to catalyze the reduction of the free aldehyde form and at the same time the oxidation of the hemiacetal structure of this class of substrates, thus leading to the occurrence of a disproportion reaction sustained by a redox recycle of the pyridine cofactor. Making use of glutathionylated alkanals devoid of the 4 hydroxyl group, and thus unable to structurally arrange into a cyclic hemiacetal form, the susceptibility to inhibition of CBR1 to polyphenols was tested. Flavones, that were much more effective than isoflavones, resulted able to modulate the reductase activity of the enzyme on this new peculiar class of substrates.

Keywords: carbonyl reductase 1, short chain dehydrogenase/reductase; 4-hydroxy-2-nonenal; 3-glutathionyl-4-hydroxyalkanals;

1. Introduction

The removal of carbonyl compounds, both from endogenous and exogenous sources, is an important step in cellular detoxification. It requires the action of several enzymes, including those belonging to the aldo-keto reductase (AKR) and short chain dehydrogenase/reductase (SDR) families [1, 2]. Among the SDR family, a prominent role is played by carbonyl reductases (CBRs), a group of NADPH-dependent enzymes [2]. Of the three CBRs in humans, encoded by three different genes, carbonyl reductase 1 (CBR1) represents the most important activity for carbonyl metabolism. CBR1 is a cytosolic monomeric protein which possesses a series of highly conserved residues, namely, Asn, Tyr, Ser and Lys. Upon hydride transfer from the cofactor NADPH, these residues enable carbonyl reduction to occur [3]. In addition, located nearby the substrate binding site, Cys227 appears to be important for the recognition and correct allocation of substrates [4]. In the CBR1 structure, a glutathione binding site has also been described, devoted to the recognition of glutathionylated substrates [5]. For the binding of this kind of substrates, Cys227 is no longer required, as demonstrated by site directed mutagenesis studies [4]. CBR1 possess a broad substrate specificity, since it is able to reduce structurally different carbonyl compounds, including xenobiotic quinones and endogenous steroids, eicosanoids and lipid-derived compounds [6-9]. In terms of physiologically occurring substrates, CBR1 has been demonstrated to intervene in the metabolism of prostaglandins, as it is able to act both as a prostaglandin 9-ketoreductase and also as a 15-hydroxyprostaglandin dehydrogenase [10]. CBR1 has also been suggested to be involved in the metabolism of the indole isatin [11]. The ability to catalyze the NADPH-dependent reduction of the lipid peroxidation product 4-oxo-nonenal (ONE) and of its glutathione adduct (GS-ONE), strengthens the role of CBR1 in antioxidant defense [9]. In addition, the ability of CBR1 to intervene in the 4-hydroxy-2-nonenal (HNE) metabolism, through the conversion of the Michael adduct of the aldehyde with glutathione (3-glutathionyl-4-hydroxynonanal, GSHNE), has been recently reported [12, 13]. Indeed, cells with impaired CBR1 activities either by siRNA or by treatment with CBR1 inhibitors have shown an increase in the level of oxidative stress markers, such as intracellular reactive oxygen species and lipid peroxidation products [14].

In cancer cells, tumor proliferation, invasion and metastasis have been demonstrated to correlate positively with an increase in CBR1 activity [15, 16]. The enzyme is also able to reduce the antitumor drugs daunorubicin and doxorubicin (DOX) into their corresponding alcohols. Several experimental studies have supported the prominent role of the enzyme in anthracycline resistance [17-19]. Indeed, the inhibition of CBR1 increases the therapeutic efficacy of DOX and reduces its cardiotoxicity, by impairing the generation of the reduction product doxorubicinol [20, 21].

This paper reports further insights on the ability of CBR1 to act on glutathionylated aldehydes, together with the ability to modulate CBR1 activity on these substrates through the action of polyphenolic compounds.

2. Materials and Methods

2.1 Materials. 9,10-phenanthrenquinone (PQ), bovine serum albumin, dimethyl sulfoxide (DMSO), D,L-dithiotreitol (DTT), reduced glutathione (GSH), hexanal, $NADP^+$, nonanal, propanal, protease inhibitors cocktail, sodium dodecyl sulphate (SDS), *trans*-2-hexenal, *trans*-2-nonenal, *trans*-2-propenal (acrolein) were purchased from Sigma–Aldrich (St. Louis, MO, USA). HHE was purchased from Cayman Chemicals (AnnArbor, MI, USA). 4*(R)*-HNE was purchased from Avanti Polar Lipids (Alabaster, AL, USA). Whatman DEAE-cellulose (DE-52) and Sephacryl S200 were purchased from GE Healthcare (Little Chalfont, UK). Blue Sepharose and Bradford reagent were purchased from Bio-Rad (Hercules, CA, USA). YM10 membranes (10 kDa cut-off) were purchased from Amicon Millipore (Darmstadt, Germany). Dialysis tubing (10 kDa cut-off) was purchased from Spectrum Laboratories (Rancho Dominguez, CA, USA). All inorganic chemicals were of reagent grade from BDH (VWR International, Poole, Dorset, UK). All solvents were HPLC grade from J.T. Baker Chemicals (Center Valley, PA, USA). NADPH was from Carbosynth (Compton, England).

2.2 Determination of CBR1 activity. Enzyme activity units were determined following the dehydrogenase activity of CBR1 at 37°C in a 50 mM sodium phosphate buffer pH 8.4 with 0.18 mM $NADP^+$ and 100 μM GSHNE as substrate. One unit of enzyme activity is defined as the amount of enzyme that catalyzes the conversion of 1 μmol of substrate in the above conditions. The reductase activity was measured at 37°C in a 50 mM sodium phosphate buffer pH 6.0 with 0.18 mM NADPH and different substrates as indicated. When the effect of polyphenols was evaluated, the assay mixture also contained 0.5% DMSO. All spectrophotometric measurements were performed at 340 nm by a Beckman DU640 spectrophotometer calibrated against air.

2.3 Expression and purification of CBR1. The expression of the human recombinant CBR1 (hCBR1) was performed as described [13]. The purification of the enzyme was carried out essentially as described for the $NADP^+$-dependent GSHNE dehydrogenase activity from a human astrocytoma cell line [12]. The pure hCBR1 (specific activity 50 U/mg) was stored at -80°C in a 10 mM sodium phosphate buffer pH 7.0 containing 0.1 mM $NADP^+$, 1.5 M NaCl, 2 mM DTT and

31% (v/v) glycerol. The enzyme was extensively dialyzed against 10 mM sodium phosphate buffer pH 7.0, before use.

2.4 Alkenals-glutathione adduct preparation. Diethylacetal of 4*(R,S)*-hydroxy-2-nonenal (HNE) was synthetized as described [12] and the free aldehyde was generated by acid hydrolysis (pH 3.0). The glutathione adducts of alkanals and alkenals were prepared by incubating GSH with different aldehydes as previously described [13].

2.5 Other methods. Protein concentration was determined according to Bradford [22] using BSA as standard protein. Statistical analysis was conducted using GraphPad software.

3. Results and Discussion

3.1 Disproportion of 3-glutathionyl-4-hydroxyalkanals. Mass spectrum analysis of GSHNE incubated with hCBR1 and substoichiometric amounts of NADPH demonstrated that the adduct possesses two functional groups recognized by the enzyme action. In fact (Fig. 1), GSHNE was able to undergo both reduction, at the level of the free aldehydic group generating 3-glutathionyl-1,4-dihydroxynonane (GSDHN), and oxidation, at the level of the hydroxyl hemiacetal group generating 3-glutathionyl-γ-nonano lactone [13]. Thus, despite the complete transformation of GSHNE, the spectrophotometric evaluation of NADPH (Fig. 2, curve 1) did not reveal any appreciable consumption of the cofactor. Essentially the same results were obtained for different NADPH concentrations (ranging from 10 to 30 μM) at different substrate/cofactor ratios (ranging from 1 to 7). The same occurred when the glutathione adduct with 4-hydroxy-2-hexenal (GSHHE) was used as substrate (Fig. 2, curve 2). On the other hand, when the aldehydic group is the only functional group susceptible to the enzyme action, as occurs with glutathione adducts with alkenals, such as GS-nonanal (Fig. 2, curves 3 and 4), a complete oxidation of the cofactor was observed. Thus hCBR1, acting both as a reductase and as a dehydrogenase on two functional groups of the same substrate, catalyzed a disproportion reaction on GSHNE, which took place through the redox recycle of the cofactor. While it was impossible to evaluate the reductase activity of hCBR1 on GSHNE, the dehydrogenase activity was easily followed by spectrophotometric evaluation at 340 nm. This was helped by the use of a basic pH to impair the reduction (an approximately 75% decrease of the reductase activity on GS-nonanal occurs going from pH 6.2 to pH 8.4), and the positive effect exerted by the predominant presence of the hemiacetal structure in solution [13].

A preferential action of hCBR1 on three of the four anomeric couples of GSHNE diastereoisomers (3-(*R,S*)GS-4-(*R,S*)HNE) generated upon spontaneous reaction between 4-(*R,S*)HNE and glutathione has been demonstrated [13]. In particular, the anomeric couple less efficiently recognized by the enzyme contained 3-(*S*)GS-4-(*R*)HNE. Furthering this evidence, the kinetic parameters were determined (at 37°C in 50 mM sodium phosphate, pH 8.4, 0.18 mM $NADP^+$ and 3.2 mU of enzyme) for the GSHNE enantiomeric couples obtained starting from 4-(*R*)HNE (3-(*R,S*)GS-4-(*R*) HNE) and compared with those previously reported for GSHNE derived from racemic HNE (13). An approximate 30% decrease in the k_{cat} value was observed using 3-(*R,S*)GS-4-(*R*) HNE as substrate (1,358 ± 52 min^{-1}), with respect to 3-(*R,S*)GS-4-(*R,S*)HNE (2,000 ± 56 min^{-1}). Conversely, no differences were observed in K_M values (27.2 ± 1.0 μM and 27.1 ± 2.8 μM, for 3-(*R,S*)GS-4-(*R,S*)HNE and 3-(*R,S*)GS-4-(*R*)HNE, respectively). It is difficult to rationalize these results, especially for the K_M values. In fact these are affected both by possible differences in the recognition of different diastereoisomers and by the different relative contribution to the mixture composition of the less active substrate. On the other hand, concerning the k_{cat} comparison, it appeared clear that the glutathionyl-4(*S*)-HNE diastereoisomers should possess a higher susceptibility to reduction with respect to the glutathionyl-4(*R*)-HNE diastereoisomers. Work is in progress to clarify this point making use of individual GSHNE diastereoisomers.
The dehydrogenase activity of hCBR1 acted not only on GSHNE, but also on GSHHE, and conceivably, on all glutathione-aldehydes adducts able to generate hemiacetal structures. Evaluation of the kinetic parameters of hCBR1 for GSHHE revealed that this substrate was recognized by the enzyme less efficiently with respect to GSHNE. In fact, while the K_M value (89.6 ± 6.9 μM) was approximately threefold higher with respect to the value measured for GSHNE [13], in the case of k_{cat} (924 ± 37 min^{-1}) a twofold decrease was observed. Thus, the decrease in the specificity constant observed for the reductase activity [13], along with the shortening of the hydrophobic chain of the substrate, was also confirmed in the case of the dehydrogenase activity.

3.2 Glutathionylated aldehydes as substrates of hCBR1. Despite the broad substrate specificity demonstrated for CBR1, aldehydes appeared to be very poor substrates for the enzyme, and their recognition by CBR1 was strictly dependent on the presence of the glutathionyl moiety. No activity was observed using propanal, nonanal, hexanal, or their corresponding *trans*-2-alkenals, as substrates of the reductase activity of hCBR1. However, the glutathionyl adducts of the above mentioned *trans*-2-alkenals were efficiently reduced by hCBR1. Figure 3 reports a comparison between 9,10 phenantrenequinone, a classical substrate of hCBR1, and GS-nonanal. From these

data, K_M values of 4.6 ± 0.4 μM and 6.5 ± 0.4 μM, and k_{cat} values of 4,002 ± 83 min^{-1} and 1,848 ± 83 min^{-1} were obtained, for PQ and GS-nonanal, respectively.

3.3 Inhibition of hCBR1 by polyphenolic compounds. Several polyphenolic compounds have been reported to affect CBR1 activity, depending on their structural features and also on the substrate used to detect the activity [23, 24]. Thus, it could be important to evaluate the effect of inhibitors on the hCBR1 catalyzed GSHNE transformation. However, the particular features of GSHNE, which allow this molecule to undergo disproportion (see Section 3.1), make the adduct not an easy manageable substrate to follow the reducing ability of CBR1. Thus GS-nonanal, the reductable substrate most similar to GSHNE, was used to test the effect of different polyphenolic compounds. Results are reported in Table 1; the resulting inhibitory pattern appeared similar, but not identical, to what previously observed using isatin as substrate of CBR1 [24]. Rutin appeared to be the most potent of the compounds tested. A comparison with quercetin indicated a positive role in determining the inhibitory effect for the rutinose moiety in position 3 of the flavonoid structure. On the other hand, no effect was exerted by the presence of rhamnose in position 3, since quercetin and quercetrin displayed essentially the same IC_{50} values. Finally, the inefficacy of isoflavons, as daidzein, daidzin and genistein in affecting CBR1 activity indicated that the movement of the hydroxyphenyl group from position 2, as in flavones, to position 3, has to be considered as the main responsible of the strongly reduced inhibitory action of these compounds. This occurred despite, as in genistein, the presence 5-hydroxy and 7-hydroxy groups, previously reported [23] to be relevant in eliciting the inhibitory potency.

The ability of CBR1 to generate GSDHN makes this enzyme potentially able to contribute to the generation of a pro-inflammatory stimulus, together with the AKR member AKR1B1, already known to be able to generate the reduced form of GSHNE [25]. As a consequence, these results open a new front in the search of anti-inflammatory molecules able to modulate NF-kB activation mediated by GSDHN. In fact, targeting AKR1B1 would not be sufficient in modulating GSDHN level. This could be strengthened by the evidence that in different cell lines treated with the AKR1B1 inhibitors Sorbinil and Tolrestat only a partial decrease of the activation of NF-kB was observed [26-28]. These observations are consistent with the ineffectiveness of Sorbinil (100 % CBR1 activity in the presence of 7 μM Sorbinil, R. Moschini, unpublished results) and Tolrestat [29] in affecting CBR1 activity.

Polyphenols are a class of secondary metabolites biosynthesized from the shikimic acid and polyacetate pathways [30] widely distributed in the plant kingdom [31]. These compounds usually exist in the form of glycosides in plants, which is the reason for their high water solubility [32]. Polyphenols have been associated with the health benefits deriving from consuming large quantities

of vegetable and fruits. Indeed, several experimental evidences demonstrate that polyphenols possess multiple biological activities, being able to act as antioxidant, anticancer and anti-inflammatory agents [33-36]. Frequently, the activity of these molecules has been associated to their inhibitory action on different enzymes [37-40]. The ability of polyphenols to inhibit CBR1 activity may clearly contribute to the overall anti-inflammatory and anticancer action of these compounds. An emblematic example may be that of epigallocatechin gallate, one of the most abundant components of green tea, a beverage whose potential as antitumoral and antioxidant has been reported [41-43]. In fact, this catechin has been demonstrated to specifically target CBR1 and enhances the action of daunorubicin in the treatment of hepatocellular carcinoma, by avoiding the generation of the cardiotoxic doxorubicinol [20].

However, CBR1 plays a relevant role also in the prostaglandin metabolism [6] and in the antioxidant defense, since its ability to remove toxic compounds as ONE, GSONE [9] and in general, as here confirmed, glutathionylated aldehydes deriving from lipid peroxidation [13, 14]. Moreover, as recently reported [44], CBR1 positively affects neuronal cell survival and confers protection against oxidative stress-induced brain degeneration. For all these, as highlighted by Bousova et al [24], the potential of polyphenols (or other naturally occurring molecules) to interfere with CBR1 activity may lead to an alteration of cell homeostasis. These considerations, together with the observed pro-oxidant action of polyphenols [45], suggest a balanced dietary intake of these molecules for healthy subjects.

4. Conclusions

In conclusion, the ability of hCBR1 to efficiently catalyze the reduction of glutathionylated aldehydes derived from lipid peroxidation, that in the case of glutathionylated-4-hydroxyalkanals is associated to the ability to oxidize the hemiacetal hydroxyl group, strengthens the pivotal role of this enzyme in cell detoxification. This confirms CBR1 as an enzymatic target whose inhibition may be relevant in favorably intervening in pathological situations.

4. Acknowledgements. This work was supported by Pisa University, PRA 2015.

5. References

[1] P. Malatkova, E. Maser, V. Wsol, Human carbonyl reductases, Curr. Drug Metab. 11 (2010) 639-658.

[2] O. A. Barski, M. S. M. Tipparaiu, A. Bhatnagar, The Aldo-Keto Reductase superfamily and its role in drug metabolism and detoxification, Drug Metab. Rev. 40 (2008) 553-624.

[3] U. Oppermann, C.Filling, M. Hult, N. Shafgat, X. Wu, M. Lindh, J. Shafgat, E. Nordling, Y. Kallberg, B. Persson, H. Jörnvall, Short-chain dehydrogenase/reductase (SDR): the 2002 update, Chem. Biol. Interact. 143-144 (2003) 247-253.

[4] J.N. Tinguely, B. Wermuth, Identification of the reactive cysteine residue (Cys227) in human carbonyl reductase, Eur. J. Biochem. 260 (1999) 9-14.

[5] R. Feldman, A. Luncsford, R.L. Heinrikson, J. Westley, J. Jarabak, Glutathione-related inhibition of prostaglandin metabolism**,** Arch. Biochem. Biophys. 211(1981) 375-381.

[6] B. Wermuth, Purification and properties of an NADPH-dependent carbonyl reductase from human brain. Relationship to prostaglandin 9-ketoreductase and xenobiotic ketone reductase, J. Biol. Chem. 256 (1981) 1206-1213.

[7] B. Wermuth, K. L. Platts, A. Seidel, F. Oesch, Carbonyl reductase provides the enzymatic basis of quinone detoxification in man, Biochem. Pharmacol. 35 (1986) 1277-1282.

[8] E.S. Pilka, F.H. Niesen, W.H. Lee, Y. El-Hawari, J. E. Dunford, G. Kochan, V. Wsol, H. J. Martin, E. Maser, U. Oppermann, Structural basis for substrate specificity in human monomeric carbonyl reductase, Plos One 4 (2009) e7113.

[9] J. A. Doorn, E. Maser, A. Blum, D. J. Claffey, D. R. Petersen, Human carbonyl reductase catalyzes reduction of 4-oxonon-2-enal, Biochemistry 43 (2004) 13106-13114.

[10] G. L. Forrest, B. Gonzalez, Carbonyl reductase Chem. Biol. Interact. 129 (2000) 21-40.

[11] N. Usami, K. Kitahara, S. Ishikura, M. Nagano, S. Sakai, A. Hara, Characterization of a major form of human isatin reductase and the reduced metabolite, Eur. J. Biochem. 268 (2001) 5755-5563.

[12] R. Moschini, E. Peroni, R. Rotondo, G. Renzone, D. Melck, M. Cappiello, M. Srebot, E. Napolitano, A. Motta, A. Scaloni, U. Mura, A. Del-Corso, NADP($^{+}$)- dependent dehydrogenase activity of carbonyl reductase on glutathionylhydroxynonanal as a new pathway for hydroxynonenal detoxification, Free Rad. Biol. Med. 83 (2015) 66-76.

[13] R. Rotondo, R. Moschini, G. Renzone, T. Tuccinardi, F. Balestri, M. Cappiello, A. Scaloni, U. Mura, A. Del-Corso, Human carbonyl reductase 1 as efficient catalyst for the reduction of

glutathionylated aldehydes derived from lipid peroxidation, Free Rad. Biol. Chem. 99 (2016) 323-332.
[14] S. Lim, J. Y. Shin, K. R. Jyothi, M. N. Nguyen, T. G. Choi, J. Kim, J. H. Park, Y. G. Eun, K. S. Yoon, J. Ha, S. S. Kim, Carbonyl reductase 1 is an essential regulator of skeletal muscle differentiation and regeneration, Int. J. Biochem. Cell Biol. 45 (2013) 1784-1793.
[15] A. Murakami, C. Fukushima, K. Yoshidomi, K. Sueoka, S. Nawata, Y. Yokoyama, S. Tsuchida, E. Ismail, F. Al-Mulla, N. Sugino, Suppression of carbonyl reductase expression enhances malignant behaviour in uterine cervical squamous cell carcinoma: carbonyl reductase predicts prognosis and lymphonode metastasis, Cancer Lett. 311 (2011) 77-84.
[16] Y. Osawa, Y. Yokoyama, T. Shigeto, M. Futagami, H. Mizunuma, Decreased expression of carbonyl reductase 1 promotes ovarian cancer growth and proliferation, Int. J. Oncol. 46 (2015) 1252-1258.
[17] M. Plebuch, M. Soldan, C. Hungerer, L. Koch, E. Maser, Increased resistanceof tumor cells to daunorubicin after transfection of cDNAs coding for anthracycline inactivating enzymes, Cancer Lett. 255 (2007) 49-56.
[18] B. Gonzalez, S. Akman, J. Doroshow, H. Rivera, W. D. Kaplan, G. L. Forrest, Protection against daunorubicin citotoxicity by expression of a cloned human carbonyl reductase cDNA in K562 leukemia cells, Cancer Res. 55 (1995) 4646-4650.
[19] W. Ax, M. Soldan, L. Koch, E. Maser, Development of daunorubicin resistance in tumour cells by induction of carbonyl reduction, Biochem. Pharmacol. 59 (2000) 293-300.
[20] W. Huang, L. Ding, Q. Huang, H. Hu, S. Liu, X. Yang, X. HU, Y. Dang, S. Shen, J. Li, X. Ji, S. Jiang, J. O. Liu, L. Yu, Carbonyl reductase 1 as a novel target of (-)-epigallocatechin gallate against hepatocellular carcinoma, Hepatology 52 (2010) 703-714.
[21] A. Jo, T. G. Choi, Y. H. Jo, K. R. Jyoyhi, M. N. Nguyen, J. H. Kim, S. Lim, M. Shahid, S. Akter, S. Lee, K. H. Lee, W. Kim, H. Ch, J. Lee, K. M. Shokat, K. S. Yoon, I. Kang, J. Ha, S. S. Kim, Inhibition of carbonyl reductase1 safely improves the efficacy of doxorubicin in breast cancer treatment, Antioxid. Redox Signal. (2016) [Epub ahead of print].
[22] M. M. Bradford, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding, Anal. Biochem. 72 (1976) 248-254.
[23] M. Carlquist, T. Frejd, M. F. Gorwa-Grauslund, Flavonoids as inhibitors of human carbonyl reductase 1, Chem Biol. Interact. 174 (2008) 98-108.
[24] I. Boušová, L. Skálová, P. Souček, P. Matoušková, The modulation of carbonyl reductase 1 by polyphenols, Drug Metab. Rev. 47 (2015) 520-533.

[25] S. Srivastava, A. Chandra, A. Bhatnagar, S.K. Srivastava, N. H. Ansari, Lipid peroxidation product, 4-hydroxynonenal and its conjugate with GSH are excellent substrates of bovine lens aldose reductase, Biochem. Biophys. Res. Commun. 217 (1995) 741-746.

[26] R. Maccari, R.M. Vitale, R. Ottanà, M. Rocchiccioli, A. Marrazzo, V. Cardile, A.C. Graziano, P. Amodeo, U. Mura, A. Del Corso, Structure-activity relationships and molecular modelling of new 5-arylidene-4-thiazolidinone derivatives as aldose reductase inhibitors and potential anti-inflammatory agents, Eur. J. Med. Chem. 81 (2014) 1-14.

[27] A. Pladzyk, A.B. Reddy, U.C. Yadav, R. Tammali, K.V. Ramana, S.K. Srivastava, Inhibition of aldose reductase prevents lipopolysaccharide-induced inflammatory response in human lens epithelial cells, Invest. Ophthalmol. Vis. Sci. 47 (2006) 5395-5403.

[28] K.V. Ramana, A. Bhatnagar, S.K. Srivastava, Inhibition of aldose reductase attenuates TNF-alpha-induced expression of adhesion molecules in endothelial cells, FASEB J. 18 (2004) 1209-1218.

[29] H.A. Gambliel, B.J. Cusack, R.E. Vestal, G.L. Forrest, R.D. Olson, Carbonyl reductase metabolizes daunorubicin (D1) to C-13 hydroxy metabolite, daunorubicinol (D2), in heart, Clin. Pharmacol. Ther. 61 (1997) 235.

[30] P. M. Dewick, Medicinal natural products, a biosynthetic approach, John Wiley & Sons, Chichester (UK), 2001.

[31] J.B. Harborne, General procedures and measurement of total phenolics, in: J. B. Harborne, P-M. Dey (Eds.), Methods in plant biochemistry, Vol. 1 Plant Phenolic, Academic Press, San Diego (CA, USA), 1990; pp. 1-29.

[32] C. Proestos, D. Sereli, M. Komaitis, Determination of phenolic compounds in aromatic plants by RP-HPLC and GC-MS, Food Chem. 95 (2006) 44-52.

[33] S. Cicerale, L. J. Lucas, R. S. Keast, Antimicrobial, antioxidant and anti-inflammatory phenolic activities in extra virgin olive oil, Curr. Opin. Biotechnol. 2 (2012) 129-135.

[34] P. Fresco, F. Borges, M. P. Marques, C. Diniz, The anticancer properties of dietary polyphenols and its relation with apoptosis, Curr. Pharm. Des. 16 (2010) 114-134.

[35] W. Guo, E. Kong, M. Meydani, Dietary polyphenols, inflammation, and cancer, Nutr. Cancer 61 (2009) 807-810.

[36] H. L. Liu, W. B. Jiang, M. X. Xie, Flavonoids: recent advances as anticancer drugs, Recent. Pat. Anticancer Drug. Discov. 5 (2010) 152-164.

[37] V. P. Androutsopoulos, A. Papakyriakou, D. Vourloumis, A. M. Tsatsakis, D. A. Spandidos, Dietary flavonoids in cancer therapy and prevention: substrates and inhibitors of cytochrome P450 CYP1 enzymes, Pharmacol. Ther. 126 (2010) 9-20.

[38] D. Lamoral-Theys, L. Pottier, F. Dufrasne, J. Neve, J. Dubois, A. Kornienko, R. Kiss, L. Ingrassia, Natural polyphenols that display anticancer properties through inhibition of kinase activity, Curr. Med. Chem. 17 (2010) 812-825.
[39] W. Guo, E. Kong, M- Meydani M, Dietary polyphenols, inflammation, and cancer, Nutr. Cancer 61 (2009) 807-10.
[40] C. Veeresham, A.R. Rao, K. Asres, Aldose reductase inhibitors of plant origin, Phytother. Res. 28 (2014) 317-333.
[41] V. M. Adhami, N. Ahmad, H. Mukhtar, Molecular targets for green tea in prostate cancer prevention, J. Nutr. 133 (2003) 2417S-2424S.
[42] R. L. Thangapazham, N. Passi, R. K. Maheshwari, Green tea polyphenol and epigallocatechin gallate induce apoptosis and inhibit invasion in human breast cancer cells, Cancer Biol. Ther. 6 (2007) 1938-1943.
[43] S. N. Tang, C. Singh, D. Nall, D. Meeker, S. Shankar, R.K. Srivastava, The dietary bioflavonoid quercetin synergizes with epigallocathechin gallate (EGCG) to inhibit prostate cancer stem cell characteristics, invasion, migration and epithelial-mesenchymal transition, J. Mol. Signal 5 (2010), doi10.1186/1750-2187-5-14.
[44] M. A. Rashid, Haque, M. Akbar, Detoxification of carbonyl compounds by carbonyl reductase in neurodegeneration, Adv. Neurobiol. 12 (2016) 355-365.
[43] S. Eghbaliferiz, M. Iranshahi, Prooxidant activity of polyphenols, flavonoids, anthocyanins and carotenoids: updated review of mechanisms and catalyzing metals, Phytother. Res. 30 (2016) 1379-1391.

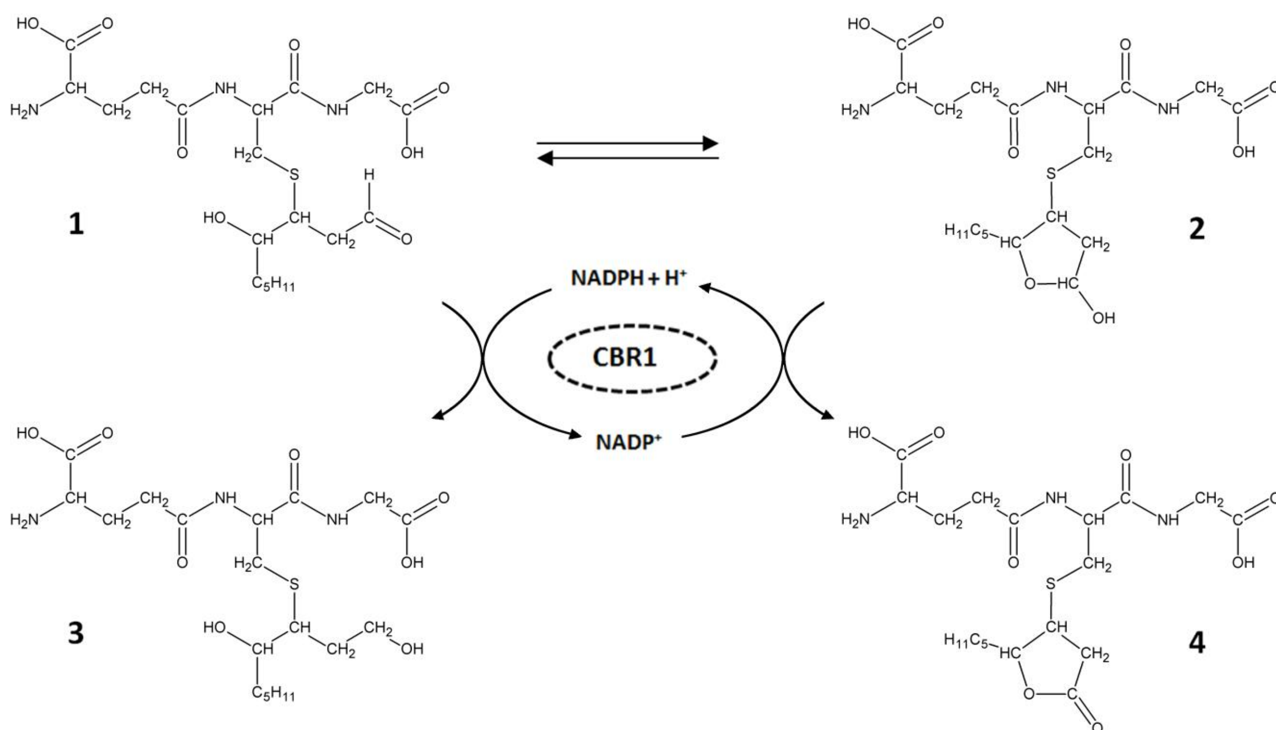

Figure 1. Schematic representation of GSHNE disproportion catalyzed by CBR1. 1: GSHNE free aldehyde; 2: GSHNE hemiacetal; 3: 3-GS-1,4-dihydroxynonane; 4: 3-GS-γ-nonano lactone.

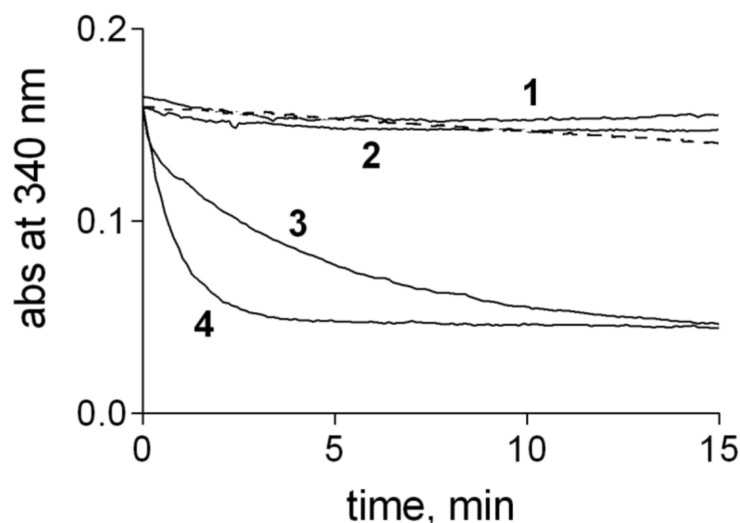

Figure 2. Time course of the hCBR1 catalyzed reduction of glutathionylated aldehydes. The absorbance at 340 nm is reported as a function of time. In a 50 mM sodium phosphate buffer pH 6.2, the assay mixtures contained 20 μM NADPH, 94 mU/ml of hCBR1 and 30 μM of either GSHNE (curve 1) or GSHHE (curve 2). Curves 3 and 4 refer to incubation mixtures as above, containing 30 μM GS-nonanal as substrate and 4 and 14 mU/ml of hCBR1, respectively. The

contribution to the decrease in absorbance due to the spontaneous NADPH degradation in the absence of substrate (dotted line) was taken into account and subtracted from each curve. Results superimposable to curves 1 and 2 were obtained when the substrate concentration was raised to 70 μM.

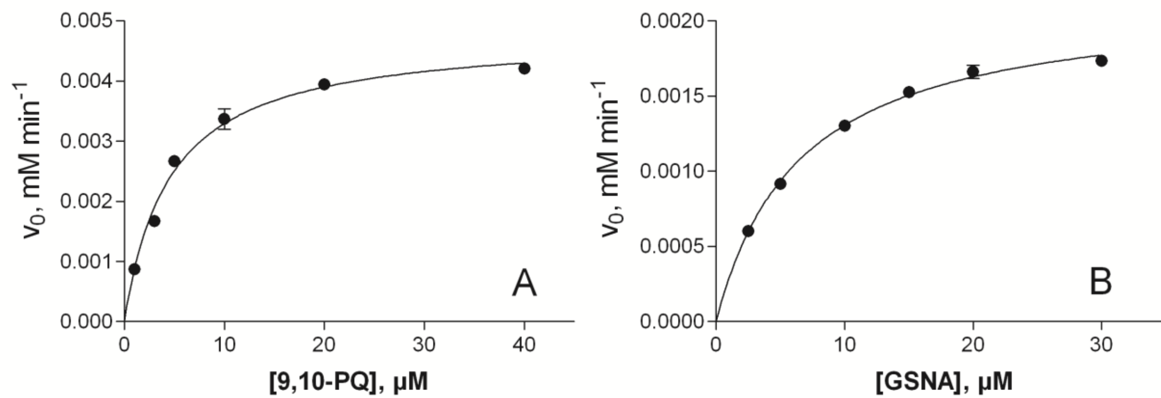

Figure 3. Kinetic analysis of the reductase activity of hCBR1. The reductase activity of hCBR1 was evaluated at 37°C in a 50 mM sodium phosphate buffer pH 6.2, containing 0.18 mM NADPH, 94 mU/ml of hCBR1 and the indicated concentrations of either 9,10 phenantrenequinone (Panel A) or GS-nonanal (GSNA) Panel B).