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Curcumin is a tight-binding inhibitor of the most efficient human daunorubicin reductase – Carbonyl reductase 1

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ABSTRACT

Curcumin is a major component of the plant *Curcuma longa* L. It is traditionally used as a spice and coloring in foods and is an important ingredient in curry. Curcuminoids have anti-oxidant and anti-inflammatory properties and gained increasing attention as potential neuroprotective and cancer preventive compounds. In the present study, we report that curcumin is a potent tight-binding inhibitor of human carbonyl reductase 1 (CBR1, K_i = 223 nM). Curcumin acts as a non-competitive inhibitor with respect to the substrate 2,3-hexandione as revealed by plotting IC_{50}-values against various substrate concentrations and most likely as a competitive inhibitor with respect to NADPH. Molecular modeling supports the finding that curcumin occupies the cofactor binding site of CBR1. Interestingly, CBR1 is one of the most effective human reductases in converting the anthracycline anti-tumor drug daunorubicin to daunorubicinol. The secondary alcohol metabolite daunorubicinol has significantly reduced anti-tumor activity and shows increased cardiotoxicity, thereby limiting the clinical use of daunorubicin. Thus, inhibition of CBR1 may increase the efficacy of daunorubicin in cancer tissue and simultaneously decrease its cardiotoxicity. Western-blots demonstrated basal expression of CBR1 in several cell lines. Significantly less daunorubicin reduction was detected after incubating A549 cell lysates with increasing concentrations of curcumin (up to 60% less with 50 μM curcumin), suggesting a beneficial effect in the co-treatment of anthracycline anti-tumor drugs together with curcumin.

1. Introduction

The yellow-orange pigment curcumin from the East Indian plant *Curcuma longa* has been used for centuries in cooking as well as in traditional Indian and Chinese medicine. A search under the keyword "curcumin" in the Pubmed database of the National Center for Biotechnology Information dates the first entry that reports its antibacterial action from 1949 [1]. Since that time numerous investigations were published describing the beneficial effects of curcumin and related compounds, mostly with respect to inflammation and cancer. Intensive investigations also led to the elucidation of a variety of biological interactions [2–4].

In this study, we present evidence that curcumin is a potent inhibitor of human carbonyl reductase type 1 (CBR1, listed as SDR21C in the SDR-database [5]), an enzyme of the short-chain dehydrogenase/reductase superfamily. CBR1 is known for more than 30 years, but even today its physiological role is not fully understood [6,7]. Expressed in many tissues, endogenous CBR1 substrates comprise steroids, eicosanoids, cofactors, neurotransmitters and polyols. In addition, a large number of xenobiotics has been identified as substrates for CBR1, including quinones, the tobacco derived carcinogen NNK (4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone) and drugs such as warfarin or ketoprofen [8–11]. Also the anthracycline anticancer drugs daunorubicin (DAUN) and doxorubicin (DOX) are reduced by CBR1, resulting in secondary alcohols at the C-13 positions (Fig. 1) [12,13].

Over time, DAUN and DOX have become a gold standard for the treatment of various cancers, such as hematological (leukemia, lymphoma) and solid breast, ovarian, lung and liver tumors [14]. Unfortunately, the clinical success of these agents is overshadowed by serious side effects, such as systemic toxicity, cardiotoxicity or drug resistance. Cardiotoxicity is the main limiting side effect that can ultimately lead to potentially lethal congestive heart failure [15].

Convincing evidence supports the idea that the C-13 hydroxy metabolites of DAUN and DOX, daunorubicinol (DAUNOL) and doxorubicinol (DOXOL), respectively, are the main trigger for chronic cardiotoxicity [16–21]. Thus, inhibition of CBR1 may increase the efficacy and decrease cardiotoxicity of anthracyclines

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[7,22,23]. Indeed, in various cell culture models it has been demonstrated that inhibition of CBR1 by known inhibitors, e.g. hydroxy-PP (3-(1-tert-butyl-4-amino-1H-pyrazolo[3,4-d]pyrimidin-3-yl)phenol), enhanced the effectiveness and decreased the cardiotoxicity of the anticancer drug DAUN by preventing its reduction to DAUNOL [24].

Here, we examined the effects of curcumin on CBR1 mediated reactions, determined its IC_{50}-value and inhibition constant K_i, and tested the inhibitory effect of curcumin on A549 cell lysates with regard to DAUNOL formation. Docking experiments were performed to study potential binding sites for curcumin and CBR1. Our results suggest that curcumin strongly inhibits CBR1-catalyzed DAUNOL formation and therefore may enhance the therapeutic effectiveness and decrease the cardiotoxic side effects of antineoplastic drugs like DAUN or DOX.

2. Materials and methods

2.1. Materials

Protein and DNA molecular weight standards were purchased from Fermentas GmbH (St. Leon-Rot, Germany). 2,3-Hexandione, curcumin and quercetin were obtained from Sigma–Aldrich (St. Louis, MO, USA). Daunorubicin was purchased from Biomol GmbH (Hamburg, Germany). NADPH, acetonitrile (gradient grade) were obtained from Carl Roth GmbH + Co. KG (Karlsruhe, Germany). Selective primary antibodies against CBR1 (Ab4148) were purchased from Abcam (Cambridge, UK), Anti-beta-actin antibody and anti-rabbit HRP-conjugated secondary antibody were obtained from Neo-Markers (Fremont, CA, USA) (Cat. No. RB-9421-P1). Cell culture media and supplements were purchased from PAA (Coelbe, Germany). Human cell lines (A549, HT-29, Caco-2, SW-480) were obtained from the Deutsche Sammlung für Mikroorganismen und Zellkulturen (DSMZ, Braunschweig, Germany) and HepG2, OVCAR-3, PANC-1, and A431 were purchased from cell lines service (CLS, Eppelheim, Germany). HCT116 cells were generously provided by J. Abel (IUF, University of Duesseldorf, Germany).

2.2. Methods

2.2.1. Preparation of recombinant carbonyl reductase 1

His-tagged human carbonyl reductase 1 (CBR1) was expressed in *Escherichia coli* and purified as published previously [25].

2.2.2. Cell culture and Western blots

Treatment of human cell lines, preparation of cell lysates and detection of CBR1 by Western blots was performed as published previously [26].

2.2.3. Inhibition of daunorubicinol formation by curcumin in A549 cell lysates

A549 cells ((3.5×10^7); 95% confluence) were rinsed 2 times with 0.1 M NaH_2PO_4 buffer (pH 7.4) and then scraped off with a cell scraper. The cells were resuspended followed by cell disruption using ultrasonication at minimal power for 10 s on ice. The protein concentration of the cell lysates was 3.6 mg/ml. Stock solutions of DAUN and curcumin were prepared in DMSO. Cell lysates (45 μg protein) were incubated for 30 min at 37 °C with 200 μM DAUN and various amounts of curcumin in a total volume of 250 μl. The final DMSO concentration did not exceed 1%. The reaction was stopped by adding 250 μl ice-cold acetonitrile and the samples were analyzed by HPLC (high-performance liquid chromatography). For DAUNOL detection a modified method of Fogli et al. was used [27] (mobile phase, 50 mM sodium phosphate/acetonitrile (75:25), pH 4.0; flow rate, 1.5 ml/min).

2.2.4. Determination of inhibition parameters

Catalytic properties were determined by measuring the decrease in absorbance at 340 nm (Cary 100 scan photometer, Varian, California, USA). A reaction mixture without inhibitor consisted of different concentrations of 2,3-hexanedione, 200 μM

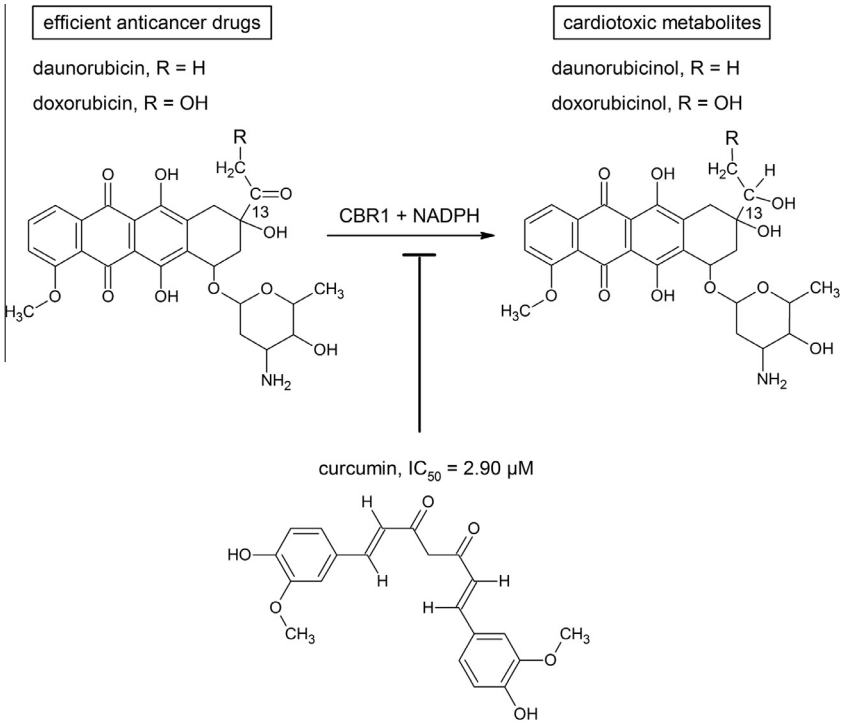

Fig. 1. Scheme of curcumin inhibition of CBR1 mediated DAUN and DOXO reduction.