Toxicology and Applied Pharmacology 293 (2016) 21–29

Contents lists available at ScienceDirect

Toxicology and Applied Pharmacology

journal homepage: www.elsevier.com/locate/ytaap

Inhibition of human anthracycline reductases by emodin — A possible remedy for anthracycline resistance

Jan Hintzpeter [a,*], Jan Moritz Seliger [a], Jakub Hofman [b], Hans-Joerg Martin [a], Vladimir Wsol [b], Edmund Maser [a]

[a] Institute of Toxicology and Pharmacology for Natural Scientists, University Medical School Schleswig-Holstein, Campus Kiel, Brunswiker Str. 10, 24105 Kiel, Germany
[b] Department of Biochemical Sciences, Faculty of Pharmacy in Hradec Kralove, Charles University in Prague, Heyrovskeho 1203, 50005 Hradec Kralove, Czech Republic

ARTICLE INFO

Article history:
Received 8 October 2015
Revised 23 December 2015
Accepted 4 January 2016
Available online 7 January 2016

Keywords:
Anthracycline resistance
Daunorubicin
Aldo-keto reductases
Short-chain dehydrogenases/reductases
Enzyme inhibition
Cardiotoxicity

ABSTRACT

The clinical application of anthracyclines, like daunorubicin and doxorubicin, is limited by two factors: dose-related cardiotoxicity and drug resistance. Both have been linked to reductive metabolism of the parent drug to their metabolites daunorubicinol and doxorubicinol, respectively. These metabolites show significantly less anti-neoplastic properties as their parent drugs and accumulate in cardiac tissue leading to chronic cardiotoxicity. Therefore, we aimed to identify novel and potent natural inhibitors for anthracycline reductases, which enhance the anticancer effect of anthracyclines by preventing the development of anthracycline resistance.
Human enzymes responsible for the reductive metabolism of daunorubicin were tested for their sensitivity towards anthrachinones, in particular emodin and anthraflavic acid. Intense inhibition kinetic data for the most effective daunorubicin reductases, including IC_{50}- and K_i-values, the mode of inhibition, as well as molecular docking, were compiled. Subsequently, a cytotoxicity profile and the ability of emodin to reverse daunorubicin resistance were determined using multiresistant A549 lung cancer and HepG2 liver cancer cells. Emodin potently inhibited the four main human daunorubicin reductases *in vitro*. Further, we could demonstrate that emodin is able to synergistically sensitize human cancer cells towards daunorubicin at clinically relevant concentrations.
Therefore, emodin may yield the potential to enhance the therapeutic effectiveness of anthracyclines by preventing anthracycline resistance via inhibition of the anthracycline reductases. In symphony with its known pharmacological properties, emodin might be a compound of particular interest in the management of anthracycline chemotherapy efficacy and their adverse effects.

1. Introduction

Anthracyclines, like daunorubicin (DAUN), doxorubicin (DOX) and related compounds, are efficient antineoplastic drugs and widely used to treat solid breast, ovarian, lung and liver tumors (Blanco et al., 2008; Hortobágyi, 1997; Lakhman et al., 2005; Licata et al., 2000; Minotti et al., 2004) as well as hematological cancers such as leukemia and lymphoma. However, their clinical use is limited by two factors: dose-related cardiotoxicity and drug resistance. Important factors that can trigger anthracycline resistance are increased enzymatic detoxification, elevated drug efflux (by ABC-transporters), interferences in apoptotic pathways and limited access to intracellular drug targets (Den Boer et al., 1998). Anthracycline related cardiotoxicity has been linked to reductive metabolism of the parent drug to their metabolites daunorubicinol (DAUNOL) and doxorubicinol (DOXOL), respectively. These secondary alcohol metabolites represent the major anthracycline metabolites in humans (see Fig. 1). Over time, the reductive metabolism has been widely accepted as one of the mechanisms that induce anthracycline resistance. The reduced anthracycline metabolites show significantly less anti-neoplastic properties as compared to their parent drug (Ax et al., 2000; Gavelová et al., 2008; Heibein et al., 2012; Kuffel et al., 1992; Schott & Robert, 1989; Soldan et al., 1996). Additionally, the reduced anthracycline metabolites accumulate in cardiac tissue over time and lead to chronic cardiotoxicity (Menna et al., 2007). For example, a 7% risk of congestive heart failure of cumulative doses of 400–450 mg/m^2 of doxorubicin have been reported for adults (Swain et al., 2003). Exposure to anthracyclines during childhood shows an increased lifetime risk of cardiotoxicity (i.e. >60% of children treated with high-dose anthracyclines) even at lower dosages (Barry et al., 2007; Lipshultz, 2006).

Members of two NADPH dependent enzyme superfamilies have been connected with reductive anthracycline metabolism: CBR1, CBR3 and CBR4 belonging to the short-chain dehydrogenase/reductase superfamily (SDR) and AKR1A1, AKR1B1, AKR1B10, AKR1C1, AKR1C2, AKR1C3 and AKR7A2 of the aldo-keto reductase superfamily (AKR) (Bains et al., 2009, 2010a,b; Hintzpeter et al., 2014; Ohara et al., 1995; Pilka et al., 2009). However, the participation of CBR3 and AKR1C2 in anthracycline metabolism is highly questionable (Hintzpeter et al., 2014; Ohara et al., 1995; Pilka et al., 2009; Takahashi et al., 2008). In case of DAUN reduction

Abbreviations: AKR, aldo-keto reductase; SDR, short-chain dehydrogenase/reductase; DAUN, daunorubicin; DOX, doxorubicin; DAUNOL, daunorubicinol; DOXOL, doxorubicinol.

* Corresponding author.
E-mail address: hintzpeter@toxi.uni-kiel.de (J. Hintzpeter).

http://dx.doi.org/10.1016/j.taap.2016.01.003
0041-008X/

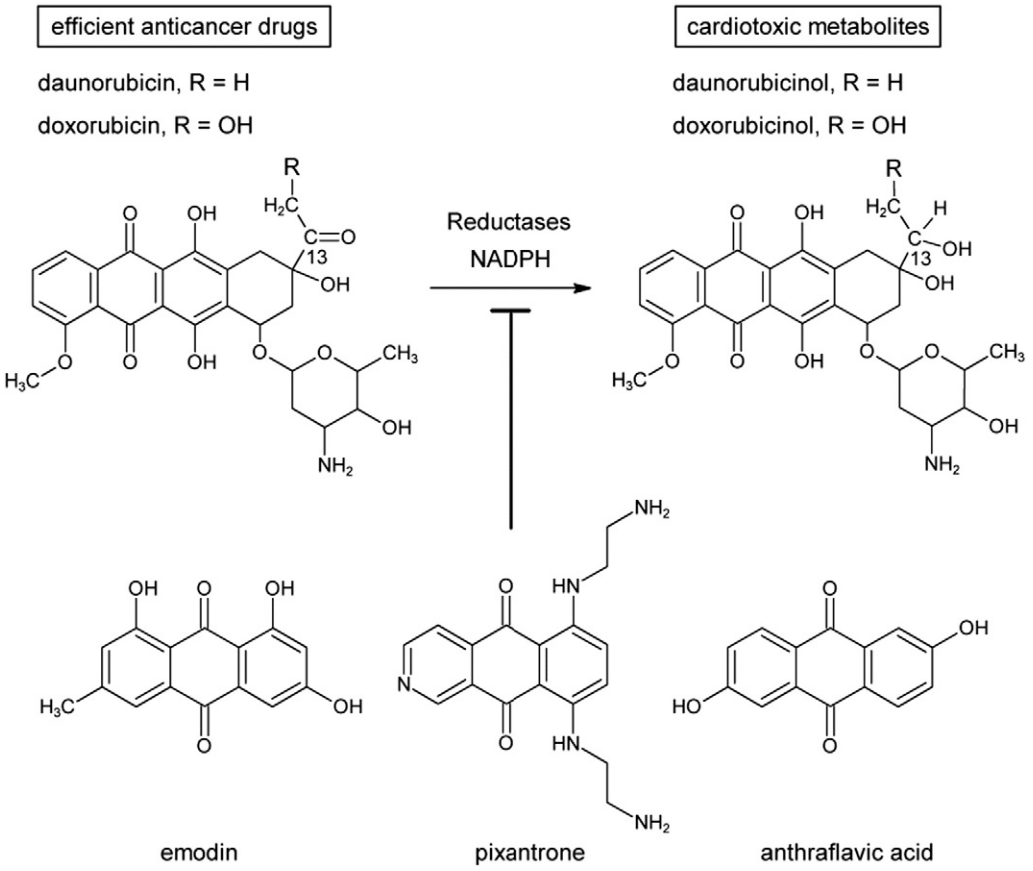

Fig. 1. Scheme of inhibition of the reductive anthracycline metabolism by emodin. The human reductases involved and their inhibitors emodin, pixantrone and anthraflavic acid are also indicated.

and regarding catalytic efficiency the most effective reductases in descending order are CBR1 (kcat/Km = 22,769 $s^{-1}\cdot M^{-1}$ (Carlquist et al., 2008) or kcat/Km = 37,800 $s^{-1}\cdot M^{-1}$ (Bains et al., 2009), AKR1C3 (kcat/Km = 8937 $s^{-1}\cdot M^{-1}$) (Bains et al., 2010a), AKR7A2 (kcat/Km = 7369 $s^{-1}\cdot M^{-1}$) (Bains et al., 2010a), AKR1B10 (kcat/Km = 2752 $s^{-1}\cdot M^{-1}$) (Bains et al., 2010a), AKR1A1 (kcat/Km = 2500 $s^{-1}\cdot M^{-1}$) (Bains et al., 2008) and AKR1B1 (kcat/Km = 329 $s^{-1}\cdot M^{-1}$) (Bains et al., 2010a), whereas the aforementioned reductases show only low (AKR1C4, AKR1C1) (Bains et al., 2010a) to (almost) none (AKR1C2, CBR3, CBR4) activity (Bains et al., 2010a; Hintzpeter et al., 2014; Pilka et al., 2009; Takahashi et al., 2008). Regarding the catalytic efficiencies towards DOX the leading enzyme is AKR1C3 (kcat/Km = 2514 $s^{-1}\cdot M^{-1}$) (Bains et al., 2010a), followed by CBR1 (kcat/Km = 720 $s^{-1}\cdot M^{-1}$) (Bains et al., 2009), AKR7A2 (kcat/Km = 457 $s^{-1}\cdot M^{-1}$) and AKR1B1 (kcat/Km = 153 $s^{-1}\cdot M^{-1}$) (Bains et al., 2010a). All other reductases are most likely to play minor roles in DOX reduction.

There is growing evidence that the C-13 hydroxy metabolites DAUNOL and DOXOL are the main trigger for chronic cardiotoxicity of anthracyclines (Bains et al., 2013; Forrest et al., 2000; Licata et al., 2000; Mordente et al., 2001; Olson et al., 1988; Propper and Maser, 1997) and, most importantly, that inhibition of specific anthracycline reductases may increase the chemotherapeutic efficacy and decrease the cardiotoxicity of anthracyclines (Cusack et al., 1993; Forrest & Gonzalez, 2000; Gavelová et al., 2008; Hofman et al., 2014; Olson et al., 2003; Tanaka et al., 2005; Zhong et al., 2011). In line with this, positive effects of the co-administration of curcumin, an inhibitor for CBR1 (Hintzpeter et al., 2014), AKR1B1 and AKR1B10 (Matsunaga et al., 2009; Muthenna et al., 2009), with DAUN or DOX have been reported in rat models (Swamy et al., 2012; Venkatesan, 1998; Venkatesan et al., 2000) and in numerous human cancer cell lines (Hosseinzadeh et al., 2011; Qian et al., 2011; Wang et al., 2011).

Interestingly, it has been shown that after pre-exposure to anthracyclines an upregulation of (eight) aldo-keto reductases and (two) carbonyl reductases (SDRs) is observed (Bains et al., 2013; Heibein et al., 2012; Hofman et al., 2014). This elevates enzymatic reduction of anthracyclines, which in turn induces drug resistance.

Taken together, these findings support the idea that the reductive metabolism of anthracyclines and its inhibition may be a promising remedy for anthracycline resistance and associated adverse effects of anthracycline treatment such as cardiotoxicity.

In the present paper, we provide evidence that emodin (6-methyl-1,3,8-trihydroxy-anthraquinone), an anthraquinone from rhubarb (*Rheum rhabarbarum*), is a potent inhibitor for four of the six most effective daunorubicin reductases. Detailed inhibition kinetic data of purified recombinant enzymes responsible for the reduction of DAUN to DAUNOL, as well as dose-dependent inhibition data from experiments with cell lysates of A549 and HepG2 cell lines that express all important anthracycline reductases in considerable amounts, support the pivotal role of these enzymes in the development of anthracycline resistance. Hence, their inhibition should be considered a possible remedy for the acquisition of drug resistance. Finally, we could demonstrate an attenuation of daunorubicin resistance by the concomitantly administered emodin in cytotoxicity tests with multiresistant A549 lung cancer and HepG2 cells at clinically relevant concentrations.

2. Material and methods

2.1. Material

2.1.1. Chemicals and reagents. Daunorubicin and emodin were purchased from Biomol GmbH (Hamburg, Germany). NADPH, acetonitrile (gradient grade) were obtained from Carl Roth GmbH + Co. KG