ELSEVIER

Biochemical Pharmacology 64 (2002) 297–305

Biochemical Pharmacology

Carbonyl reduction of the potential cytostatic drugs benfluron and 3,9-dimethoxybenfluron in human *in vitro*

Lenka Skálová[a], Milan Nobilis[b], Barbora Szotáková[a], Eliška Kondrová[a], Michal Šavlík[a], Vladimír Wsól[a], Lydiane Pichard-Garcia[c], Edmund Maser[d,*]

[a]*Department of Biochemical Sciences, Faculty of Pharmacy, Charles University, Research Centre LN00B12, Heyrovského 1203, CZ-500 05 Hradec Králové, Czech Republic*

[b]*Institute of Exp. Biopharmaceutics of the Academy of Sciences of the Czech Republic, Heyrovského, CZ-500 05 Hradec Králové, Czech Republic*

[c]*INSERM U-128, CNRS, 1919 Route de Mende, 34033 Montpellier, France*

[d]*Department of Pharmacology and Toxicology, School of Medicine, Philipps University of Marburg, Karl-von-Frisch-Strasse 1, D-35033 Marburg, Germany*

Received 14 January 2002; accepted 17 April 2002

Abstract

Benfluron (B, [5-(2-*N*-oxo-2-*N*′,*N*″-dimethylaminoethoxy)-7-oxo-7*H*-benzo[*c*]fluorene]) is a potential benzo[*c*]fluorene antineoplastic agent with high activity against a broad spectrum of experimental tumors *in vitro* and *in vivo*. The structure of B has been modified to repress its rapid deactivation through carbonyl reduction on C7. 3,9-Dimethoxybenfluron (D, [3,9-dimethoxy-5-(2-*N*-oxo-2-*N*′,*N*″-dimethylaminoethoxy)-7-oxo-7*H*-benzo[*c*]fluorene]) is one of the B derivatives developed. The present paper was designed to compare the C7 carbonyl reduction of B and D in microsomes, cytosol and hepatocytes from human liver. Two purified human enzymes, microsomal 11β-hydroxysteroid dehydrogenase 1 (11β-HSD 1) and cytosolic carbonyl reductase, were tested if they are responsible for B and D carbonyl reduction in the respective fractions. Indeed, carbonyl reduction of D in comparison to that of B was 4 and 6–10 times less extensive in human liver microsomes and cytosol, respectively. Moreover, about 10–20 times higher amounts of dihydro B than dihydro D were detected in primary culture of human hepatocytes. 11β-HSD 1 was shown to be able to reduce B and D. For this enzyme, about 10 times higher rates of carbonyl reduction were observed for B than for D. Likewise, CR participates in B and D carbonyl reduction, although smaller amounts of both reduced metabolites were detected. In summary, carbonyl reduction of D was significantly less extensive than that of B in all *in vitro* experiments. This lower rate of D inactivation was especially pronounced in hepatocytes which represent a close to *in vivo* situation. Our results clearly demonstrate that dimethoxy substitution protects the carbonyl group of the benzo[*c*]fluorene moiety against the deactivation by microsomal and cytosolic reductases. Detailed knowledge on the participating enzymes may serve as a basis for the co-application of specific inhibitors in chemotherapy to further improve the pharmacokinetics of benzo[*c*]fluorene derivatives.

Keywords: Chemotherapy; Drug resistance; 11β-Hydroxysteroid dehydrogenase; Carbonyl reductase; Structure–metabolism relationship; Human hepatocytes

1. Introduction

The metabolism of drugs has been extensively studied with regard to their oxidation. Less attention has been paid to their reductive metabolism, although it is now well known that a variety of pharmacologically important substances undergo reductive reactions in their biotransformation [1]. Carbonyl reduction is a significant step in the phase I biotransformation of a great variety of aromatic, alicyclic and aliphatic carbonyl compounds, including pharmacologically relevant substances [2,3]. Carbonyl reduction has also been shown to be of significance in various inactivation processes of drugs containing a carbonyl group, such as warfarin, haloperidol, daunorubicin and doxorubicin [4,5].

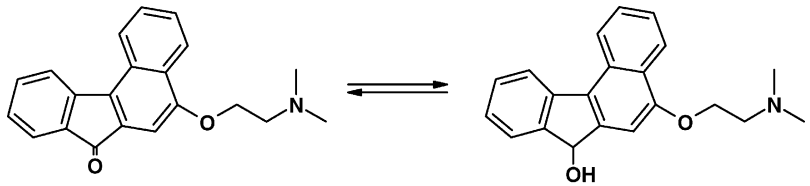
Several enzymes mediating carbonyl reduction of compounds from endogenous and exogenous origin have been identified until now, and most of them are characterized on

* Corresponding author. Tel.: +49-6421-28-65465; fax: +49-6421-28-65600.

E-mail address: maser@mailer.uni-marburg.de (E. Maser).

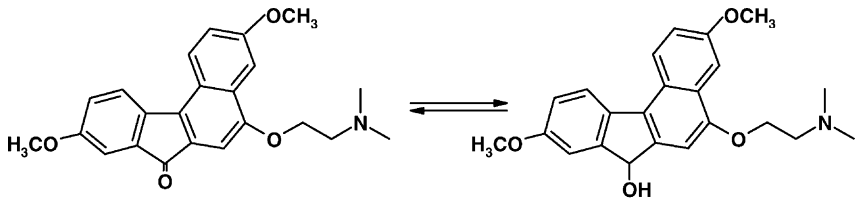
Abbreviations: B, benfluron, [5-(2-*N*-oxo-2-*N*′,*N*″-dimethylaminoethoxy)-7-oxo-7*H*-benzo[*c*]fluorene]; D, 3,9-dimethoxybenfluron [3,9-dimethoxy-5-(2-*N*-oxo-2-*N*′,*N*″-dimethyl aminoethoxy)-7-oxo-7*H*-benzo[*c*]fluorene]; dhB, 7-dihydrobenfluron; dhD, 7-dihydro-3,9-dimethoxybenfluron; 11β-HSD 1, 11β-hydroxysteroid dehydrogenase 1; CR, carbonyl reductase.

0006-2952/02/$ – see front matter
PII: S0006-2952(02)01068-7



benfluron (B) 7-dihydrobenfluron (dhB)

Fig. 1. Chemical structure of benfluron (B) and its metabolite 7-dihydrobenfluron (dhB).



3,9-dimethoxybenfluron (D) 7-dihydro-3,9-dimethoxybenfluron (dhD)

Fig. 2. Chemical structure of 3,9-dimethoxybenfluron (D) and its metabolite 7-dihydro-3,9-dimethoxybenfluron (dhD).

the biochemical and molecular level [6]. According to their primary structure and conserved amino acid residue motifs, these enzymes have been classified into two protein superfamilies, the short-chain dehydrogenases (SDRs) [7] and the aldo–keto reductases (AKRs) [8]. The enzymes occur in every tissue and organism examined so far and some of them appear to have their physiological role in the metabolism of prostaglandins, sugars and steroids [7,9]. Interestingly, some hydroxysteroid dehydrogenases exhibit pluripotency in that they catalyze the carbonyl reduction of xenobiotic aldehydes and ketones, in addition to being specific towards their physiological steroid substrates [5].

Deactivation through carbonyl reduction is also considered to be the principle biotransformation way of the potential cytostatic drug benfluron (Fig. 1). Benfluron (B), an antineoplastic agent prepared in the Research Institute for Pharmacy and Biochemistry in Prague, exhibits a high activity against a broad spectrum of experimental tumors *in vitro* and *in vivo* [10]. A DNA intercalation mode of action was proposed (unpublished data).

In the rat, the main metabolic pathways of B both *in vivo* and *in vitro* are reduction of the carbonyl group at C7, *N*-oxidation, *N*-demethylation, as well as hydroxylation on C9 [11–13]. In human, dihydrobenfluron (dhB) formed by carbonyl reduction represents the principle metabolite of B (unpublished data). In recent years, the chemical structure of B has been modified with the aim to repress its disadvantageous pharmacokinetic properties (rapid deactivation through carbonyl reduction on C7, low accessibility from the gastrointestinal tract and strong binding to tissue proteins). 3,9-Dimethoxybenfluron (D) is one of the B derivatives developed (Fig. 2). High antineoplastic efficiencies of D can be expected *in vivo* [14,15] and respective studies are currently in progress.

Our study was designed to quantitatively evaluate and compare the extent of B and D carbonyl reduction in microsomes, cytosol and hepatocytes from human liver. In a further approach, two enzymes isolated from human liver, microsomal 11β-HSD 1 (EC 1.1.1.146) and cytosolic carbonyl reductase (CR, EC 1.1.1.184), were identified as responsible for some B and D carbonyl reduction. The data obtained are promising in that dimethoxy substitution at C3 and C9 considerably protects D from deactivation by carbonyl reduction at C7.

2. Materials and methods

2.1. Chemicals

The alkylation reagent 2-chlorethyldimethylamine used in benzo[*c*]fluorene synthesis was purchased from Sigma. NADPH was from Boehringer and HPLC chemicals from Merck. The B precursor, 5-hydroxy-7-oxo-7*H*-benzo[*c*]-fluorene, was obtained from the Research Institute for Pharmacy and Biochemistry, Prague (Czech Republic), the phenolic precursor for D (5-hydroxy-3,9-dimethoxy-7-oxo-7*H*-benzo[*c*]fluorene) was prepared in our laboratories. All other chemicals were of the highest purity commercially available.

2.2. Syntheses of benzo[c]fluorene derivatives

The benzo[*c*]fluorene derivatives B and D were prepared by the Williamson synthesis from corresponding phenols and basic alkylation reagents. TLC and HPLC standards of the corresponding C7 dihydro-metabolites were prepared by a $NaBH_4$ mediated reduction of parent compounds in methanol solution. The structure and the purity of the

compounds were confirmed by NMR and by HPLC coupled with spectra analysis.

2.3. Biological material

The human liver samples were obtained from the Cadaver Donor Programme of Transplant Centre of Faculty of Medicine, Charles University, Hradec Králové.

2.4. Preparation of subcellular fractions

The liver homogenate was prepared by homogenization in a Potter and Elvehjem homogenizer in a ratio of 1:3 (w/v) in a 0.1 M sodium phosphate buffer, pH 7.4, and by sonication with Sonopuls (Bandeline). The microsomal and cytosolic fractions were isolated by fractional ultracentrifugation of the liver homogenate in the same buffer. Microsomes were finally resuspended in a buffer containing 20% glycerol (v/v) and stored at −80°.

2.5. Preparation of hepatocytes

Hepatocytes from two donors (68-year-old male and 48-year-old male) were prepared in INSERM U128 laboratories (France). Hepatocytes from the third donor (69-year-old female) were prepared in Faculty of Pharmacy, Charles University (Czech republic). The two-step collagenase perfusion method [16] modified according to [17,18] was used. Three million cells, in a volume of 3 mL culture medium, were placed into 60 mm plastic dishes pre-coated with collagen. The culture medium consisted of a 1:1 mixture of Ham's F12 and William's E, supplemented as described in [19]. The fetal calf serum was added to the culture medium (5%) to favor the attachment of the cells during the first 4 hr after plating. Thereafter, the medium was changed in the absence of serum. The cultures were maintained at 37° in a humidified atmosphere of air and 5% CO_2.

2.6. Activity assays with subcellular fractions and hepatocytes

Suitable conditions for activity tests (substrate and cosubstrate concentrations, time of incubation) were chosen according to preliminary experiments. The microsomal suspension or cytosol (0.1 mL corresponding to 1 mg of protein in 0.1 M Na–phosphate buffer, pH 7.4) was incubated with 0.6 μmol of co-substrates (a mixture of NADPH and NADH) and with the substrates at a saturating concentration of 1.5 mM. The incubations (total volume of 0.3 mL) were carried out at 37° for 30 min under aeration or argon atmosphere.

Hepatocyte monolayers, cultured on a Petri dish (diameter 60 mm) at a density of 0.8–1 million cells/mL medium as described by [20] were incubated with 100 μM of substrate dissolved in the culture medium. In these cases, low substrate concentrations were used to escape the toxic effects of the substrates on cells. Aliquots of medium (0.5 mL) were collected in the intervals of 0, 1, 3, 6 and 24 hr or 0, 2, 4, 8 and 24 hr.

All incubations were terminated by addition of 0.1 mL of 26% aqueous ammonia solution or 1 M sodium hydroxide. The samples were extracted three times with 2 vol. of distilled ethyl acetate and the combined extracts were vacuum-evaporated to dryness. The evaporation residues were dissolved in methanol prior to TLC analyses or in mobile phase prior to HPLC injection.

2.7. Activity assays with purified 11β-HSD 1 and CR

11β-HSD 1 was isolated from human liver according to Maser *et al.* [21]. CR was a generous gift from Ursula Breyer-Pfaff, University of Tübingen (Germany) [22]. Standard incubation mixtures with 5, 10 or 20 μL of native human 11β-HSD 1 (protein concentration: 71 μg/mL) or native human CR (protein concentrations: 47 μg/mL) were incubated with 10 μL of a NADPH-regenerating system (final concentrations: 0.8 mM $NADP^+$, 6 mM glucose 6-phosphate, 0.35 U glucose 6-phosphate dehydrogenase, 3 mM $MgCl_2$) in 100 mM sodium phosphate buffer, pH 7.4, and B or D (0.2 mM or 1.0 mM) as the substrate in a total buffer volume of 50 μL (0.1 M Na–phosphate buffer, pH 7.4). After a reaction time of 30 min at 37°, all incubations were terminated by cooling to 0° and addition of 20 μL of 26% aqueous ammonia solution. Metabolites were extracted three times with 2 vol. of distilled ethyl acetate, and the combined extracts were evaporated in vacuum until dryness. The dry evaporation residues were dissolved in the mobile phase prior to their HPLC injection.

2.8. Protein determination

Protein concentration was determined using the Lowry method with 0.1% SDS.

2.9. TLC analyses

Benfluron or dimethoxybenfluron and their corresponding metabolites were separated on TLC plates (Si 60 F254, Merck). The mobile phase consisted of methanol, chloroform, and triethylamine in the ratio of 10:75:4 (v/v/v). Quantification of substances was carried out with the help of a Camag TLC Scanner II, and the UV detection was performed in dual wavelength mode (295 and 340 nm for B and its metabolites; 315 and 365 nm for D and its metabolites).

2.10. HPLC separation and detection

HPLC analyses were performed using a *Thermo Separation Products* (formerly Spectra Physics) chromatograph.

The chromatographic system consisted of a SCM400 solvent degasser, P4000 quaternary gradient pump, AS 3500 autosampler with 100 μL sample loop, SpectraFOCUS high-speed scanning UV–VIS detector, SN4000 system controller and a data station with the analytical software ChromQuest 2.1 (ThermoQuest Inc.). A LiChroCART 125–4 mm analytical column packed with Purospher RP-18e (5 μm) and a LiChroCART 4–4 mm precolumn with the same stationary phase (Merck) were used for the analyses. The mobile phase consisted of 0.01 M nonylamine buffer (pH 7.4), acetonitrile, and propan-2-ol (in the ratio of 2:2:1, v/v/v). The flow rate was 1 mL × min^{-1}. The UV detection was performed either in dual wavelength mode (295 and 340 nm for B and its metabolites; 315 and 365 nm for D and its metabolites) or in high-speed scanning mode (range 195–365 nm with 1 nm steps), used for UV spectra collection.

3. Results

3.1. Reduction of B and D in subcellular fractions

In vitro metabolism of B and D was studied in subcellular fractions of human liver. Carbonyl reduction of B and D was found to occur in both the microsomal as well as the cytosolic fraction. The results are shown in Table 1.

Compared to substrate B, very low activities of carbonyl reduction of D were found in both subcellular fractions. For example, the formation of 7-dihydro-3,9-dimethoxybenfluron (dhD) was 4 and 6–10 times less extensive than that of dhB in microsomes and cytosol, respectively. Carbonyl reduction of B in human liver predominated in cytosol as compared to microsomes. Higher amounts of dihydro-metabolites were found in samples incubated under anaerobic conditions than under aerobic conditions.

3.2. Reduction of B and D in human hepatocytes

Primary human hepatocytes from three donors were used for investigating the formation of dihydro-metabolites from B or D as substrates. Fig. 3 shows the yield of dhB and dhD in the culture medium during increasing time intervals. Although absolute levels of metabolite formation varied among the individual donors, the overall course of substrate reduction was similar in all hepatocyte preparations. Most interestingly, considerable differences were found between levels of dhB and dhD formation. In all the time intervals tested, 10–20 times higher rates of carbonyl reduction were observed with B than with D as the substrate.

Table 1
Specific activities of enzymes involved in carbonyl reduction of benfluron (B) and dimethoxybenfluron (D) in subcellular fractions of human liver

Substrate	Activity of reductases (nmol × mg^{-1} × 30 min^{-1})			
	Cytosol		Microsomes	
	Aerobic	Anaerobic	Aerobic	Anaerobic
B	51 ± 2	64 ± 5	23 ± 2	37 ± 3
D	5 ± 1	10 ± 3	6 ± 1	9 ± 2

The values represent the mean ± SD of three samples. Both NADH plus NADPH served as co-substrates. See Section 2 for experimental procedures and details.

3.3. 11β-HSD 1 and CR are responsible for carbonyl reduction of B and D

Previous investigations have shown that microsomal 11β-HSD 1 and cytosolic CR are important enzymes in the detoxification of xenobiotic carbonyl compounds. Therefore, both enzymes isolated from human liver were tested in their purified form if they can catalyze the carbonyl reduction of B and D.

Substrates B and D (in 0.2 and 1 mM concentration) were incubated with three different amounts of purified human 11β-HSD 1. Fig. 4 shows the yield of dhB or dhD formed by 11β-HSD 1 mediated carbonyl reduction. The linear dependence between product formation and enzyme amount proved the ability of 11β-HSD 1 to catalyze the carbonyl reduction of both B and D. Interestingly, about 10 times higher rates of dhB production were observed compared to dhD.

Likewise, substrates B and D (in 0.2 and 1 mM concentration) were tested with three different amounts of purified human CR. The results are presented in Fig. 5. Although the production of dihydro-metabolites was low (5–10 times lower yields than those observed for 11β-HSD 1), it could clearly been shown that CR is able to participate in B and D carbonyl reduction. Comparing the two substrates, a significantly higher formation of dhB than of dhD by purified CR was observed, as was the case with purified 11β-HSD 1.

4. Discussion

Reduction of carbonyl group containing substances, especially cytostatic drugs, is of great interest, since the resulting hydroxy-metabolites are considered to be far less toxic for the tumor cells [23–25]. Accordingly, the prevention of carbonyl reduction may represent a potential approach to enhance the safety and efficiency of cytostatics in clinical chemotherapy [26]. Prevention of carbonyl reduction could be achieved either by inhibiting the enzymes responsible for drug inactivation or by chemically modifying the chemotherapeutic molecule, such that it loses its affinity for the inactivating enzymes.

Several examples of an altered reductive metabolism after structural modification of the substrates have been reported [4,6,27,28]. We previously studied the effects

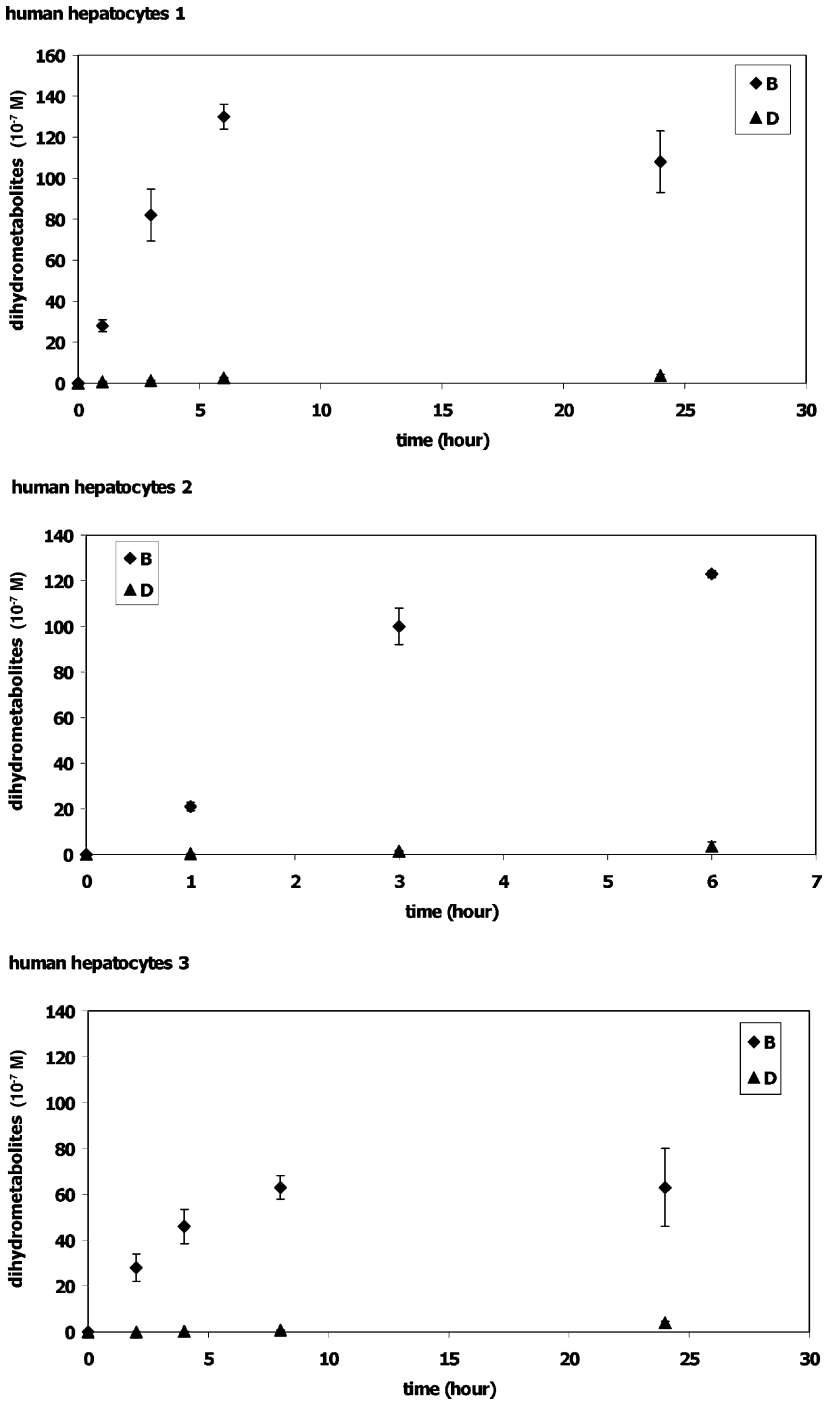


Fig. 3. Formation of dhB and dhD through carbonyl reduction of benfluron (B) and dimethoxybenfluron (D) in primary cultures of human hepatocytes from three different donors. Concentrations of substrate (B or D) and the corresponding dihydro-metabolite (dhB or dhD) were measured in culture medium aliquots. Each value represents the mean ± SD of three samples. See Section 2 for experimental procedures and details.

of substituents on reduction of benzo[*c*]fluorene *N*-oxides [29]. Based on a comparison of the amount of dihydro-metabolites of individual *N*-oxides, an important effect of dimethoxy substitution has been found. As no dihydro-metabolites of dimethoxybenfluron *N*-oxide were formed in rat microsomes, we suppose that the dimethoxy substitution in the benzo[*c*]fluorene ring causes the effective C7 carbonyl group protection in rat microsomes.

Rapid deactivation through carbonyl reduction represents one of the undesired properties of B. Its structure has been modified with an effort to decrease the metabolic conversion mainly to 7-dihydrobenfluron (dhB), as it is considered to be the most important deactivation product of B [10–12,30]. Our present study was aimed to verify this hypothesis in human liver on several experimental levels.

At first, B and D carbonyl reduction was studied in microsomes and cytosol from human liver. To provide

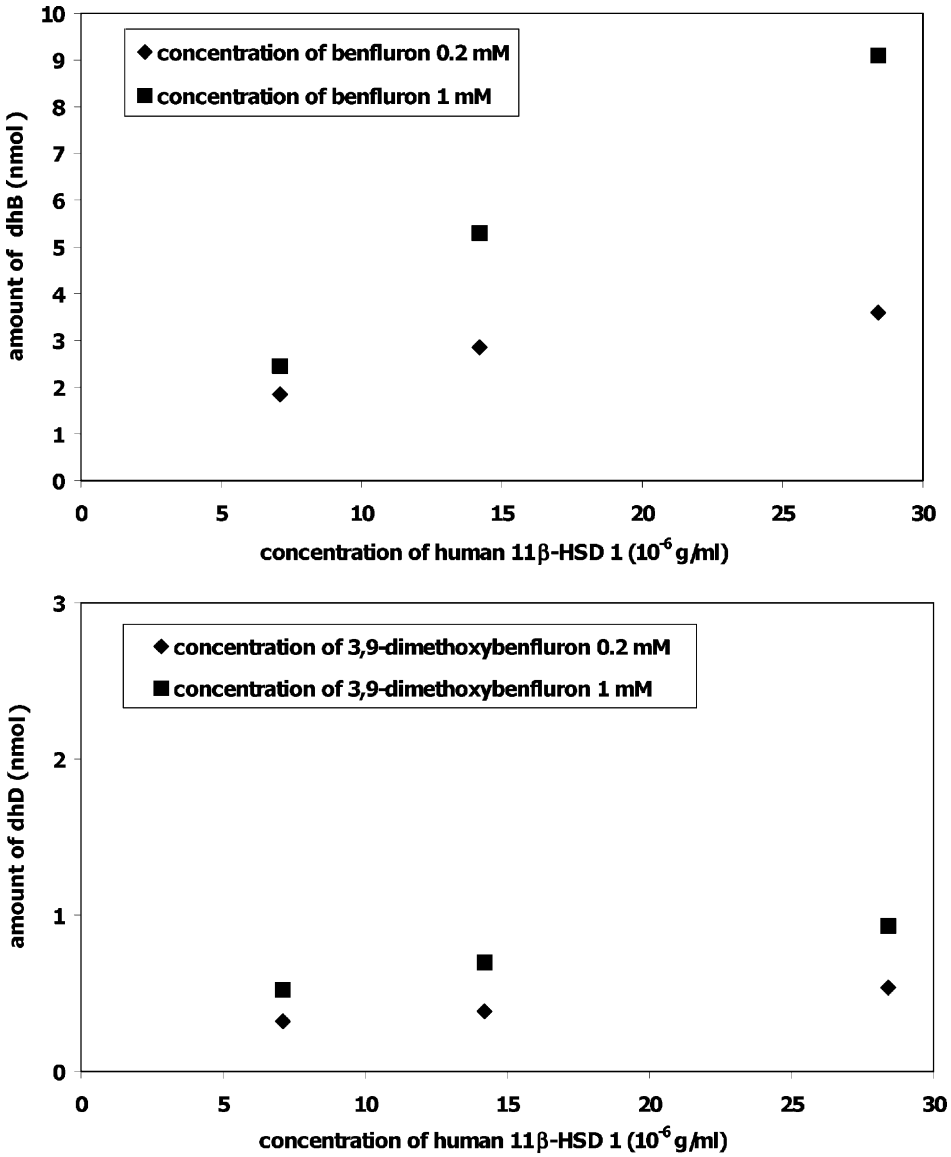


Fig. 4. The amount of dhB and dhD formed by carbonyl reduction of benfluron (B) and dimethoxybenfluron (D) in incubation mixtures with purified human 11β-HSD 1 in the presence of a NADPH-regenerating system. Each value represents the mean of two samples. See Section 2 for experimental procedures and details.

co-substrates for all reductases possibly involved, and to minimize the usage of human material, a mixture of NADH and NADPH was used. Experiments under anaerobic as well as under aerobic conditions were carried out, because of some reductase activities that could be affected by oxygen [1]. In addition, anaerobic conditions simulate hypoxic conditions in the central part of solid tumors [31]. Our benzo[*c*]fluorene substrates were reduced in both subcellular fractions, which is in contrast to daunorubicin [26] and some other carbonyl containing drugs, e.g. naltrexone [32] and timiperone [33] that are reduced only in cytosol. Our results indicate that at least two enzyme systems (one microsomal and one cytosolic) catalyze the carbonyl reduction of B and D. Both microsomal and cytosolic enzyme systems unambiguously preferred substrate B than substrate D. No dramatic increase of reduction of B and D under anaerobic compared to aerobic conditions was observed. These results agree with our previous data obtained in rat liver subcellular fractions (data not shown). However, in contrast to human liver, carbonyl reduction of B predominated in rat liver microsomes compared to rat liver cytosol.

To study a close to *in vivo* situation, B and D carbonyl reduction was then investigated in human hepatocytes with low substrate concentrations and without NADH or NADPH supplementation. The resulting kinetic data are very promising. Whereas B carbonyl reduction in hepatocytes was significant, that of D was largely negligible. Dimethoxy substitution to protect the carbonyl moiety of the benzo[*c*]fluorenes against deactivation by reductases seems to be an important approach to improve the pharmacokinetics of these chemotherapeutics. On

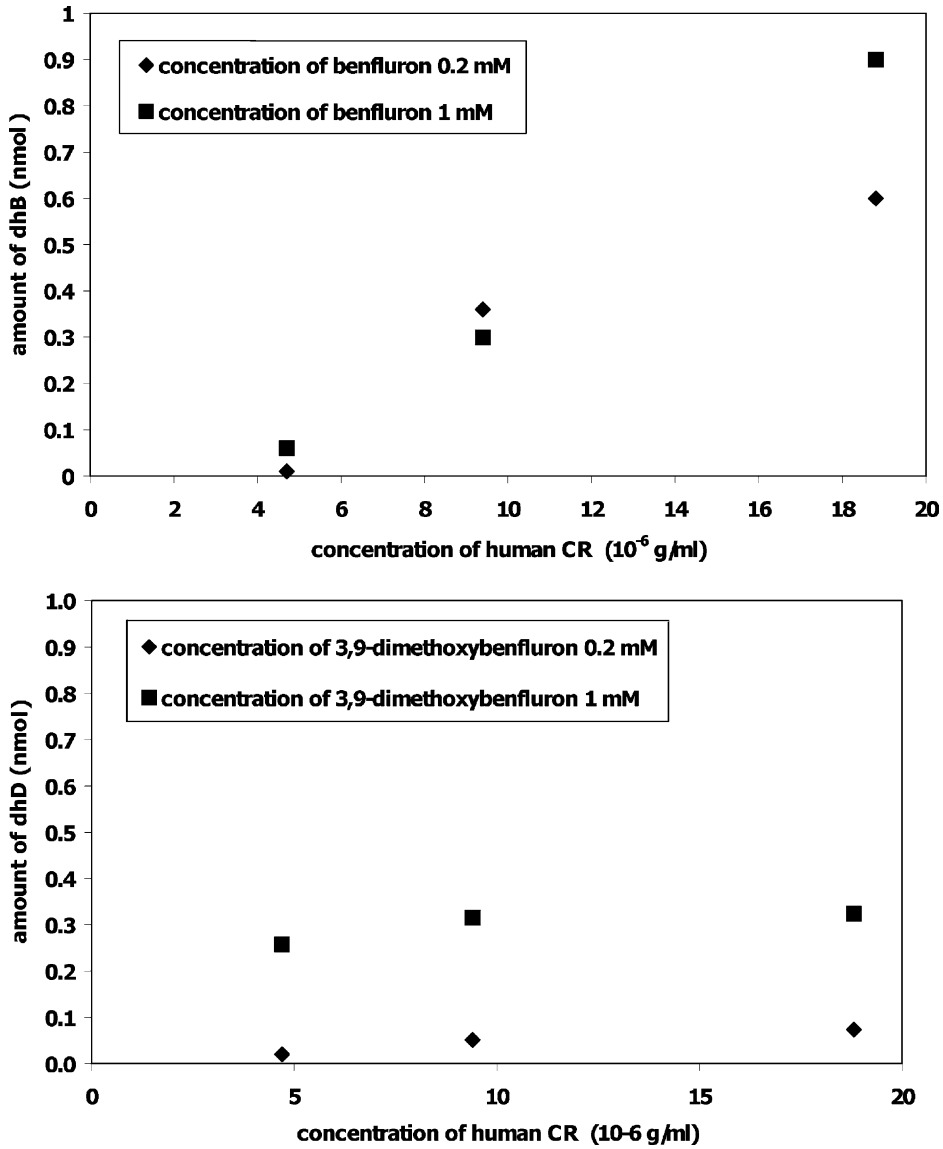


Fig. 5. The amount of dhB and dhD formed by carbonyl reduction of benfluron (B) and dimethoxybenfluron (D) in incubation mixtures with purified human CR in the presence of a NADPH-regenerating system. Each value represents the mean of two samples. See Section 2 for experimental procedures and details.

the other hand, a partial role of possible lower cell permeability for D than B (data are not available yet) to decrease metabolism of D in hepatocytes cannot be excluded.

Finally, two candidate enzymes for B and D carbonyl reduction, isolated from human liver, were investigated. The physiological role of microsomal 11β-HSD 1 is the reversible interconversion of active (cortisol) into inactive (cortisone) glucocorticoids [34]. This enzyme was also shown to be important for the microsomal carbonyl reduction of non-steroidal xenobiotics [35–37], and was therefore tested with B and D in the present study. A specific physiological role for CR is currently not known, however, this NADPH-dependent enzyme serves as the major carbonyl reducing enzyme in cytosolic fractions [38]. For example, CR has been shown to catalyze the carbonyl reduction of chemotherapeutics (daunorubicin, doxorubicin) and a large number of other biologically and pharmacologically active carbonyl compounds [25].

In our experiments, both 11β-HSD 1 and CR were shown to metabolize B and D by carbonyl reduction. Compared to 11β-HSD 1, the formation of dihydroxy-metabolites by CR was low. The participation of other cytosolic reductases in B and D biotransformation can thus not be excluded, especially with respect to the high reducing activities in human liver cytosol. Importantly, in all cases, carbonyl reduction of B was higher than that of D on the level of purified enzymes, thereby supporting the concept of protecting benzo[*c*]fluorenes from inactivation by dimethoxy substitution. Moreover, the identification of the enzymes participating in D inactivation may serve as a basis for the simultaneous application of

specific inhibitors (e.g. glycyrrhetinic acid for 11β-HSD 1 [39]; quercitrin for CR [38]) to further improve the pharmacokinetics of benzo[*c*]fluorene derivatives in chemotherapy.

5. Conclusions

The activities of enzymes reducing the carbonyl group of B and D were compared on several experimental levels. The formation of dhD was significantly less extensive than formation of dhB in all *in vitro* experiments. These results verify our hypothesis that dimethoxy substitution protects the carbonyl group of the benzo[*c*]fluorene moiety against the activity of reductases. A remarkable restriction of D carbonyl reduction compared to that of B was especially observed in the primary culture of human hepatocytes. With regard to these observations, a low extent of carbonyl reduction of D can be expected *in vivo*. Introduction of two methoxy groups seems to be a promising way of preventing the metabolic inactivation of B. The favorable pharmacokinetic properties of D compared to B are advantageous in the development of D as a potent chemotherapeutic, as are information on the participating enzymes and their specific inhibitors.

Acknowledgments

Authors are grateful to the Transplant Centre (Hradec Králové) for providing human liver samples. Special thanks to Patrick Maurel (INSERM U128, CNRS, Montpelliere, France) for generously giving us the opportunity to work in his laboratory. The project was supported by the Czech Ministry of Education (MSM111600002) and by the Deutsche Forschungsgemeinschaft (MA 1704/3-2).

References

[1] Testa B. The metabolism of drugs and other xenobiotics. Biochemistry of redox reactions. London: Academic Press, 1995.
[2] Felsted R, Bachur NR. Mammalian carbonyl reductases. Drug Metab Rev 1980;11:1–60.
[3] Wermuth B. Aldo–keto reductases. Prog Clin Biol Res 1985;174: 209–30.
[4] Hermans JJR, Thijssen HHW. Properties and stereoselectivity of carbonyl reductases involved in the ketone reduction of warfarin and analogues. Adv Exp Med Biol 1993;328:351–60.
[5] Maser E. Xenobiotic carbonyl reduction and physiological steroid oxidoreduction. Biochem Pharmacol 1995;49:421–40.
[6] Oppermann UCT, Nagel G, Belai I, Bueld JE, Genti-Raimondi S, Koolman J, Netter KJ, Maser E. Carbonyl reduction of an anti-insect agent imidazole analogue of metyrapone in soil bacteria, invertebrate and vertebrate species. Chem Biol Interact 1998;114: 211–24.
[7] Jörnvall H, Persson B, Krook M, Atrian S, Gonzalez-Duarte R, Jeffery J, Ghosh D. Short-chain dehydrogenases/reductases (SDR). Biochemistry 1995;34:6003–13.
[8] Bohren KM, Bullock B, Wermuth B, Gabbay KH. The aldo–keto reductase superfamily cDNAs and deduced amino acid sequences of human aldehyde and aldose reductases. J Biol Chem 1989;264: 9547–51.
[9] Jez JM, Penning TM. The aldo–keto reductase (AKR) superfamily: an update. Chem Biol Interact 2001;130–132:499–525.
[10] Miko M, Krepelka J, Melka M. Effects of benfluron and its two metabolites on respiratory processes in P388 murine leukemia and Ehrlich ascites cells. Biochem Pharmacol 1991;42:214–6.
[11] Kvasnicková E, Nobilis M, Hais IM. Chromatographic characterization of *in vitro* metabolites of 5-[2-(*N*,*N*-dimethylamino)ethoxy]-7-oxo-7*H*-benzo[*c*]fluorine. J Chromatogr 1984;295:201–9.
[12] Nobilis M, Kvasnicková E, Šroler A, Hais IM. Elimination of benflurone and its metabolites in the faeces and urine of rats. Drug Metab Drug Interact 1991;9:225–40.
[13] Hrubý K, Anzenbacherová E, Anzenbacher P, Nobilis M. Potential cancerostatic benfluron is metabolized by peroxidase: *in vitro* biotransformation of benfluron by horseradish peroxidase. Gen Physiol Biophys 1997;16:321–7.
[14] Janotová S, Horáková K. 9-Hydroxybenfluron induced inhibition of proliferation metabolism in HeLa cells. Cell Biochem Funct 1993;11:131–5.
[15] Miko M, Krepelka J, Melka M. 9-Hydroxybenfluron: cytostatic effects and inhibition of macromolecular synthesis in Ehrlich ascites and P388 murine leukemia cells. Anticancer Drugs 1991;2:289–95.
[16] Berry MN, Edwards AM, Barritt GJ. Isolated hepatocytes: preparation, properties and applications. Laboratory techniques in biochemistry and molecular biology. Amsterdam: Elsevier, 1991.
[17] Pichard L, Fabre I, Domergue J, Saint-Aubert B, Mourad G, Maurel P. Cyclosporin A drug interaction. Screening for inducers and inhibitors of cytochrome P450 in primary culture of human hepatocytes and in liver microsomes. Drug Metab Dispos 1990;18:595–606.
[18] Pichard-Garcia L, Hyland R, Baulieu J, Fabre JM, Milton A, Maurel P. Human hepatocytes in primary culture predict lack of cytochrome P450 3A4 induction by eletriptan *in vivo*. Drug Metab Dispos 2000;28:51–7.
[19] Isom HC, Georgoff I. Quantitative assay for albumine-producing liver cell after simian virus transformation of rat hepatocytes maintained in chemically defined medium. Proc Natl Acad Sci USA 1984;8: 6378–82.
[20] Maurel P. The use of adult human hepatocytes in primary culture and other *in vitro* systems to investigate drug metabolism in man. Adv Drug Deliv Rev 1996;22:105–32.
[21] Maser E, Völker B, Friebertshäuser J. 11β-Hydroxysteroid dehydrogenase type 1 from human liver: dimerization and enzyme cooperativity support its postulated role as glucocorticoid reductase. Biochemistry 2001;41:2459–65.
[22] Breyer-Pfaff U, Nill K. High-affinity stereoselective reduction of the enantiomers of ketotifen and of ketonic nortriptyline metabolites by aldo–keto reductases from human liver. Biochem Pharmacol 2000;59:249–60.
[23] Ax W, Soldan M, Koch L, Maser E. Development of daunorubicin resistance in tumor cells by induction of carbonyl reduction. Biochem Pharmacol 2000;59:293–300.
[24] López de Cerain A, Marín A, Idoate MA, Tuñón MT, Bello J. Carbonyl reductase and NADPH cytochrome P450 reductase activities in human tumoral vs. normal tissues. Eur J Cancer 1999;35:320–4.
[25] Forrest GL, Gonzalez B. Carbonyl reductase. Chem Biol Interact 2000;129:21–40.
[26] Pröpper D, Maser E. Carbonyl reduction of daunorubicin in rabbit liver and heart. Pharmacol Toxicol 1997;80:240–5.
[27] Ritter CL, Decker RW, Malejka-Giganti D. Reductions of nitro and 9-oxo groups of environmental nitrofluorenes by the rat mammary gland *in vitro*. Chem Res Toxicol 2000;13:793–800.
[28] Yoshimoto A, Tobe H, Johdo O, Ishikura T, Takeuchi T. Structure-sensitivity relationship of anthracycline antibiotics to C7-reduction by redox enzymes. Jpn J Antibiot 1991;44:287–95.

[29] Skálová L, Nobilis M, Szotáková B, Wsól V, Kubícek V, Baliharová V, Kvasnicková E. Effect of substituents on microsomal reduction of benzo[*c*]fluorene *N*-oxides. Chem Biol Interact 2000;126:185–200.
[30] Miko M, Krepelka J, Melka M, Vajdová D. Effect of benfluron metabolites on ATP level in tumor cells. Neoplasma 1989;36:411–7.
[31] Overgaard J. Sensitization of hypoxic tumor cells: clinical experience. Int J Radiat Biol 1989;56:801–11.
[32] Porter J, Somogyi AA, White JM. Kinetics and inhibition of the formation of 6β-naltrexol from naltrexone in human liver cytosol. Br J Clin Pharmacol 2000;50:465–71.
[33] Shimoda K, Shibasaki M, Inaba T, Cheung SW, Someya T, Takahashi S. Carbonyl reduction of timiperone in human liver cytosol. Pharmacol Toxicol 1998;83:164–8.
[34] Seckl JR, Walker BR. Minireview: 11β-hydroxysteroid dehydrogenase type 1: a tissue-specific amplifier of glucocorticoid action. Endocrinology 2001;142:1371–6.
[35] Maser E, Oppermann UCT. Role of type 1 11β-hydroxysteroid dehydrogenase in detoxification processes. Eur J Biochem 1997;249: 365–9.
[36] Maser E. 11β-Hydroxysteroid dehydrogenase type 1: another steroid metabolizing enzyme involved in detoxication processes. Curr Top Steroid Res 1998;1:37–57.
[37] Oppermann UCT, Maser E. Molecular and structural aspects of xenobiotic carbonyl metabolizing enzymes. Role of reductases and dehydrogenases in xenobiotic phase I reactions. Toxicology 2000;144:71–81.
[38] Wermuth B, Bohren KM, Heinemann G, von Wartburg JP, Gabbay KH. Human carbonyl reductase. Nucleotide sequence analysis of a cDNA and amino acid sequence of the encoded protein. J Biol Chem 1988;263:16185–8.
[39] Seckl JR. 11β-Hydroxysteroid dehydrogenase in the brain: a novel regulator of glucocorticoid action? Front Neuroendocrinol 1997; 18:49–99.