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Stereospecific reduction of the original anticancer drug oracin in rat extrahepatic tissues

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Abstract

The liver is the major site of drug metabolism in the body. However, many drugs undergo metabolism in extrahepatic sites and in the gut wall and lumen. In this study, the distribution and activity of reductases in rat that reduced potential cytostatic oracin to its principal metabolite 11-dihydrooracin (DHO) were investigated. The extension and stereospecificity of oracin reduction to DHO were tested in microsomal and cytosolic fractions from the liver, kidney, heart, lung and wall of small intestine, caecum and large intestine. Intestinal bacterial reduction of oracin was studied as well. The amount of DHO enantiomers was measured by HPLC with Chiralcel OD-R as chiral column. Reductive biotransformation of oracin was mostly stereospecific for (+)-DHO, but the enantiomeric ratio differed significantly among individual tissues and subcellular fractions (from 56% (+)-DHO in heart microsomes to 92% (+)-DHO in liver cytosol). Stereospecificity for (−)-DHO (60%) was observed in bacterial oracin reduction in the lumen of small intestine, caecum and large intestine. Shift of the (+)-DHO/(−)-DHO enantiomeric ratio from 90:10 (in liver subcellular fractions) to 60:40 (in-vivo) clearly demonstrated the importance of the contribution of extrahepatic metabolism to the total biotransformation of oracin to DHO.

Introduction

NADPH-dependent enzymes (e.g. carbonyl reductases, aldo-keto reductases) mediate carbonyl reductions of pharmacologically active xenobiotic carbonyl compounds to the corresponding alcohols (Wermuth et al 1988, Jez & Penning 2001). Their high specific activity is expressed predominantly by the cytosolic fractions but the membraneous fractions also contain a considerable amount of carbonyl reductase activity (Oppermann et al 1991).

Many endogenous compounds, such as prostaglandins, biogenic amines and steroids, as well as xenobiotic aromatic and aliphatic aldehydes and ketones, are converted to the corresponding alcohols before their further metabolism or elimination (Felsted & Bachur 1980). The new cytostatic drug oracin contains the carbonyl group and undergoes carbonyl reduction as a route of deactivation. Oracin, 6-[2-(2-hydroxyethyl) aminoethyl]-5,11-dioxo-5,6-dihydro-11H-indeno[1,2-c] isoquinoline (Michalský 1992) (Figure 1), is a promising cytostatic drug (Melka 1993), which is already in phase II of clinical trials. The planar structure of oracin is typical for a DNA intercalation mode of action and also inhibition of topoisomerase I and II was proven (Miko et al 2000, 2002). The possibility of peroral administration, the combination of different antitumour mechanisms (Melka 1999), the absence of cardiotoxicity (Gersl et al 1996) and the negative results in the Ames test on mutagenicity (Marhan 1995) are the main advantages of this novel chemotherapeutic. Due to its promising biological activity in chemotherapy and favourable pharmacokinetic properties, biotransformation of oracin is being intensively studied.

The principal in-vivo and in-vitro metabolite of oracin in all laboratory animals studied, as well as in man, is 11-dihydrooracin, 6-[2-(2-hydroxyethyl)aminoethyl]-5-oxo-11-hydroxy-5,6-dihydro-11H-indeno[1,2-c] isoquinoline (DHO) (Wsól et al 1996, 1998, 2000; Szotáková et al 1996). This chiral metabolite is formed by reduction of the

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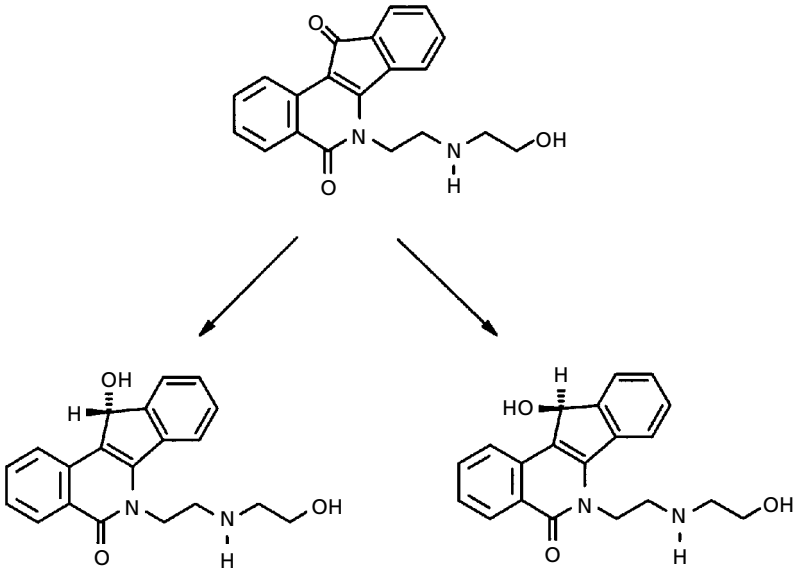

Figure 1 Reduction of oracin to (+)-DHO and (−)-DHO enantiomers.

pro-chiral carbonyl group at the 11-position of the oracin molecule. Microsomal reductases possess stereospecificity in DHO formation and strong effect of species, age and gender on this stereospecificity was observed (Wsol et al 1999, Skalová et al 1999). 11β-hydroxysteroid dehydrogenase type 1 and CYP1A seem to participate in oracin carbonyl reduction at the microsomal level in rat liver. In the same study carbonyl reductase is postulated to be one of the enzymes responsible for DHO formation in rat liver cytosol (Szotáková et al 2000).

While present knowledge about oracin biotransformation in liver has been sufficient, no relevant information about metabolism of oracin in extrahepatic tissues has been available.

Metabolism in the gut lumen and wall can decrease the bioavailability and the pharmacological effects of a wide variety of drugs (Ilett et al 1990). Intestinal bacteria are generally involved in the reduction of a variety of compounds possessing double bonds, aldehyde, ketone, alcohol, N-oxide, nitro and azo groups (Kashiyama et al 1994). Bacterial biotransformation is the most extensive in the colon, while gut wall metabolism is generally highest in the jejunum and decreases distally. Enzyme activity is associated primarily with the mucosal epithelial cells. Since all orally administered drugs must pass through these cells during absorption, biotransformation may occur if suitable enzymes are present (Ilett et al 1990). Several reviews refer to a wide variety of metabolic reactions (both phase I and II) occurring in the gut wall and also a wide range of enzymes that can metabolise drugs and other xenobiotics (e.g. Back & Rogers 1987; Ilett et al 1990; Krishna & Klotz 1994; Johnson et al 2000; Fisher et al 2001).

In the kidney, drug metabolising enzymes are present in both the renal cortex and the renal medulla. CYP 2E1, CYP 2B and CYP 3A apoproteins were determined by Western blot analysis in male rats (Ronis et al 1998). Carbonyl reductases (Parkinson 2001) and aldo-keto reductases (O'Connor et al 1999) are able to reduce certain alcohols and ketones in the kidney. The distribution of P450 enzymes in the lung is similar to that in the kidney. The presence of aldo-keto reductases in lung is evident but not so high as in kidney (O'Connor et al 1999). The presence of various xenobiotic-metabolising enzymes in other organs, tissues (e.g. brain, skin, heart) and plasma of animals and man has been demonstrated (Krishna & Klotz 1994; Behnia & Boroujerdi 1999).

The purpose of this study was to evaluate and compare the extension and stereospecificity of oracin reduction to DHO in microsomal and cytosolic fractions from the liver, kidney, heart, lung and gut wall. The contribution of intestinal bacteria from small intestine, caecum and large intestine to overall oracin reduction was also evaluated.

Materials and Methods

Chemicals

Oracin, (+)-DHO and (−)-DHO were obtained from the Research Institute for Pharmacy and Biochemistry (Prague, Czech Republic). Coenzyme NADPH was purchased from Sigma-Aldrich (Prague, Czech Republic). Acetonitrile and methanol (both HPLC grade) were obtained from Merck (Prague, Czech Republic), sodium perchlorate monohydrate and perchloric acid were from Fluka (Prague, Czech Republic). Medium for cultivation of gut microbes (thioglycolate broth; Imuna, Slovakia) was prepared immediately before use. All other chemicals were of analytical grade.

Animals and biological materials

Male Wistar rats (10–12 weeks) were obtained from BioTest (Konárovice, Czech Republic). Rats were cared for and used in accordance with the Guide for the Care and Use of Laboratory Animals (Protection of Animals from Cruelty Act. no. 246/92, Czech Republic).

Liver, kidney, lung, heart and caecum and the small and large intestinal wall of rats were used as a source of microsomes and cytosol. Tissues were homogenised in 0.1 M sodium phosphate buffer, pH 7.4. The microsomal and cytosolic fractions were obtained by fractional ultracentrifugation of the homogenate (Gillette 1971). Obtained cytosol was stored at −80 °C. A re-washing step (followed by second ultracentrifugation) was added at the end of the microsomal preparation procedure. Microsomes were finally re-suspended in the homogenization buffer containing 20% glycerol (v/v) and stored at −80 °C. Protein was determined according to the modified method of Lowry with SDS (Markwell et al 1978).

For in-vitro investigation of metabolism caused by intestinal microbes, samples of intestinal content were withdrawn from distal ileum (2 cm from ileocaecal valve), from caecum (the middle of curvature) and from colon ascendens (2 cm from ileocaecal valve) by means of a bacteriological loop after aseptic incision. The pool (approximately 100 μL) obtained from 3 rats was inoculated into thioglycolate medium and cultivated in anaerobic jars (Oxoid). After 24 h cultivation at 37 °C under anaerobic conditions, the media with bacteria from the individual intestinal sections were pipetted (10 mL) into sterile test tubes containing 0.5 mL oracin stock solution of various concentrations.

For in-vivo studies, urine and faeces were collected 48 h after oral administration of oracin (150 mg kg^{-1}).

Incubation, extraction and stability of DHO enantiomers

Standard incubation mixtures were prepared in Eppendorf microtubes. The microsomal suspension (100 μL, corresponding to 0.1 g wet tissue) or cytosolic fraction (100 μL, corresponding to 0.02 g wet tissue) were incubated with 1 mM oracin and 2 mM NADPH in a total buffer volume of 0.3 mL. Incubations at 37 °C under aeration or in argon atmosphere were performed for 30 min. All incubations were terminated by cooling to 0 °C and adding 26% aqueous ammonia solution to pH 10.8–11.0 and extracted three times with double volumes of distilled ethyl acetate; combined extracts were evaporated to dryness in vacuum. The dry samples were dissolved in the mobile phase before their HPLC injection.

Intestinal microflora from the small intestine, caecum and large intestine of rats were anaerobically cultivated (37 °C) in liquid medium (10 mL) for 0, 2, 8, 24 and 72 h with oracin (final concentration 0.2 mM), or for 24 h with various concentrations of oracin (20, 50, 100, 150, 200, 250 and 300 μM). The cultivation was finished in different time intervals by cooling and adding aqueous ammonia (26%) to pH 10.8–11.0, extracted twice with distilled ethyl acetate and combined extracts were evaporated to dryness in vacuum.

Stability study of individual DHO enantiomers in 0.1 M sodium phosphate buffer, (pH 7.4 and 11.0) and in mobile phase for 24 h and 7 days proved that there was no non-enzymatic chiral inversion for both DHO enantiomers.

High performance liquid chromatography

The HPLC system consisted of a Spectra Series P200 gradient pump from Spectra-Physics (Fremont, CA), an HP 1100 Series autosampler, an HP 1100 Series thermostatted column compartment from Hewlett Packard (Waldbronn, Germany), a multichannel UV–Vis detector PU4021 from Pye Unicam (Cambridge, England) and a fluorescence detector PU4027 (Cambridge, UK). DHO was detected by fluorescence detector using an excitation wavelength of 340 nm and an emission wavelength of 418 nm. Oracin was detected by UV–Vis detector at a detection wavelength of 280 nm. Data from chromatographic runs were processed using a chromatography station for Windows CSW (version 1.7) software from DataApex (Prague, Czech Republic) on a PII/233 PC from AutoCont (Hradec Králové, Czech Republic). Separation of enantiomers was performed using a 250 × 4.6 mm ODR Chiralcel column. The mobile phase was prepared by mixing buffer (0.3 M sodium perchlorate, pH 3.00 set by $HClO_4$) with acetonitrile (69:31 v/v). HPLC separation was performed at 25 °C with a flow rate of 0.5 mL min^{-1}.

Statistical analysis

In the study of oracin biotransformation in subcellular fractions, the one-way analysis of variance was performed for comparison of specific activity of oracin-reducing enzymes in the organs tested and of the effect of aerobic or anaerobic conditions to DHO production. Data from six individual incubations ($n = 6$) were compared.

Results

Reduction of oracin in liver, kidney, lung and heart subcellular fractions

The activity of oracin reductases was measured in liver, kidney, lung and heart microsomes and cytosol. Results are shown in Table 1. The activitiy of oracin reductases in extrahepatic tissues was comparable with the liver oracin reductases. The specific activity was in some cases even significantly higher (heart and kidney cytosol, lung microsomes ($P < 0.05$–0.001; Table 1). The reductive biotransformation of oracin in liver, kidney, lung and heart was stereospecific. In all these tissues significantly more (+)-DHO was formed (both in aerobic and anaerobic conditions, except for heart microsomes in anaerobic conditions) and the DHO enantiomer ratio differed. The lowest amount of (+)-DHO was observed in heart

Table 1 Specific activity and stereospecificity of oracin-reducing enzymes in rat liver, kidney, lung and heart microsomes and cytosol.

		Activity (nmol/30 min/mg)		(+)-DHO:(−)-DHO ratio
		(+)-DHO	(−)-DHO	
Liver	M air	0.38 ± 0.03	0.04 ± 0.02	9.50
	M argon	0.45 ± 0.04	0.08 ± 0.02	5.63
	C air	0.34 ± 0.01	0.03 ± 0.00	10.11
	C argon	0.39 ± 0.03	0.05 ± 0.01	7.80
Kidney	M air	0.30 ± 0.07	0.17 ± 0.04*	1.78
	M argon	0.63 ± 0.03**	0.18 ± 0.01**	3.55
	C air	0.49 ± 0.02**	0.19 ± 0.01***	2.57
	C argon	0.64 ± 0.11*	0.18 ± 0.03**	3.55
Lung	M air	1.56 ± 0.37*	0.77 ± 0.27*	2.13
	M argon	3.39 ± 0.33***	0.56 ± 0.05***	6.14
	C air	0.37 ± 0.19	0.12 ± 0.04	3.00
	C argon	0.71 ± 0.21	0.19 ± 0.05*	3.76
Heart	M air	0.09 ± 0.00***	0.05 ± 0.01	1.86
	M argon	0.10 ± 0.00***	0.08 ± 0.01	1.33
	C air	0.57 ± 0.09*	0.17 ± 0.03**	3.35
	C argon	1.67 ± 0.34*	0.41 ± 0.08**	4.00

M, microsomes; C, cytosol. Values represent the average of six experiments (n = 6); *P < 0.05, **P < 0.01, ***P < 0.001 vs mean values in liver.

microsomes (56%) and the highest in liver cytosol (92% (+)-DHO) (Table 1).

Reduction of oracin in gut wall microsomes and cytosol

Incubation of oracin with the rat intestinal microsomal and cytosolic fractions confirmed that DHO is also reduced in the gut wall. Results are presented in Table 2. The specific activity of oracin-reducing enzymes in the gut wall is comparable with the activity of liver oracin reductases. Significantly higher (P < 0.05–0.001; Table 2) specific activity was found in the cytosolic fraction of all parts of the gut wall than in liver cytosol. On the other hand, gut wall microsomes produced a significantly lower amount of (+)-DHO than did the liver microsomal fraction under aerobic conditions. The specific activity of microsomal reductases in the intestinal wall was found to be lower than in the cytosolic intestinal fractions. Reductive biotransformation of oracin in gut wall was stereospecific as

Table 2 Specific activity and stereospecificity of oracin-reducing enzymes in different parts of the rat gut wall.

		Activity (nmol/30 min/mg)		(+)-DHO:(−)-DHO ratio
		(+)-DHO	(−)-DHO	
Small intestine	M air	0.25 ± 0.02**	0.24 ± 0.01***	1.00
	M argon	0.39 ± 0.01	0.28 ± 0.02***	1.33
	C air	1.99 ± 0.24***	0.87 ± 0.48	2.57
	C argon	3.42 ± 0.19***	0.77 ± 0.04***	4.56
Caecum	M air	0.29 ± 0.02*	0.29 ± 0.02***	1.00
	M argon	0.61 ± 0.00**	0.46 ± 0.00***	1.33
	C air	1.81 ± 0.10***	1.07 ± 0.14***	1.70
	C argon	2.89 ± 0.07***	0.80 ± 0.00***	3.76
Large intestine	M air	0.25 ± 0.01***	0.21 ± 0.01***	1.17
	M argon	0.38 ± 0.01	0.20 ± 0.00***	1.94
	C air	0.58 ± 0.02***	0.21 ± 0.01***	2.85
	C argon	1.11 ± 0.22**	0.27 ± 0.06**	4.00

Values represent the average of six experiments (n = 6). M, microsomes; C, cytosol. *P < 0.05, **P < 0.01, ***P < 0.001 vs mean values in liver.

well. Both microsomal and cytosolic fractions of the small intestine, caecum and large intestine produced significantly more (+)-DHO, except for microsomes from the small intestinal and caecum wall under aerobic conditions. The high stereospecificity of oracin reductases was found in intestinal cytosol under anaerobic conditions, where 82%, 80% and 78% of (+)-DHO was detected in the small intestine, large intestine and caecum, respectively. The microsomal fraction from the wall of the small and large intestines and caecum formed both DHO enantiomers in the same amount. The DHO enantiomers ratio did not change significantly (Table 2).

Bacterial reduction of oracin

Intestinal bacteria were incubated anaerobically with oracin in-vitro. Cultivation of intestinal microflora from the small intestine, caecum and large intestine of male rats with oracin revealed that bacteria from all parts of the gut are able to reduce this anticancer drug significantly (Table 3). The highest oracin-reducing activity was found in bacteria from rat caecum, and the lowest was found in bacteria from the small intestine.

Bacterial carbonyl reducing enzymes produced different amounts of the two DHO enantiomers. Here, the formation of (−)-DHO predominated over that of (+)-DHO. Production of individual enantiomers of DHO in the lumen of the small intestine, caecum and large intestine differed only a little. The amounts detected were 58% (−)-DHO in small intestine, 62% (−)-DHO in caecum and 54% (−)-DHO in large intestine (Table 3). The time dependence of DHO enantiomer formation was also investigated. In anaerobic cultivation of the microflora of the small intestine, caecum and large intestine with oracin, more (−)-DHO than (+)-DHO was produced in all time periods (not shown).

The concentration dependence of oracin carbonyl reduction in bacterial cultures was studied over a range of substrate concentrations (0.02–0.30 mM; Figure 2) with the aim of evaluating the inhibitory influence of oracin on gut microflora. The caecum microflora of rat was chosen, due to the highest population of the main strains of intestinal bacteria (Smith 1965; Hawksworth et al 1971). This dependence was not linear — a concentration of 0.25 and 0.30 mM oracin in the cultivation medium inhibited bacterial oracin reduction.

Table 3 Activity and stereospecificity of oracin-reducing enzymes in rat gut lumen (in-vitro incubation of intestinal microflora).

	(+)-DHO (μg)	(−)-DHO (μg)	(+)-DHO:(−)-DHO ratio
Small intestine	2.76 ± 0.71	3.78 ± 0.75	0.73
Caecum	3.67 ± 0.69	5.94 ± 0.92	0.62
Large intestine	3.26 ± 0.80	3.88 ± 0.53	0.84

Values represent the average of four experiments (n= 4).

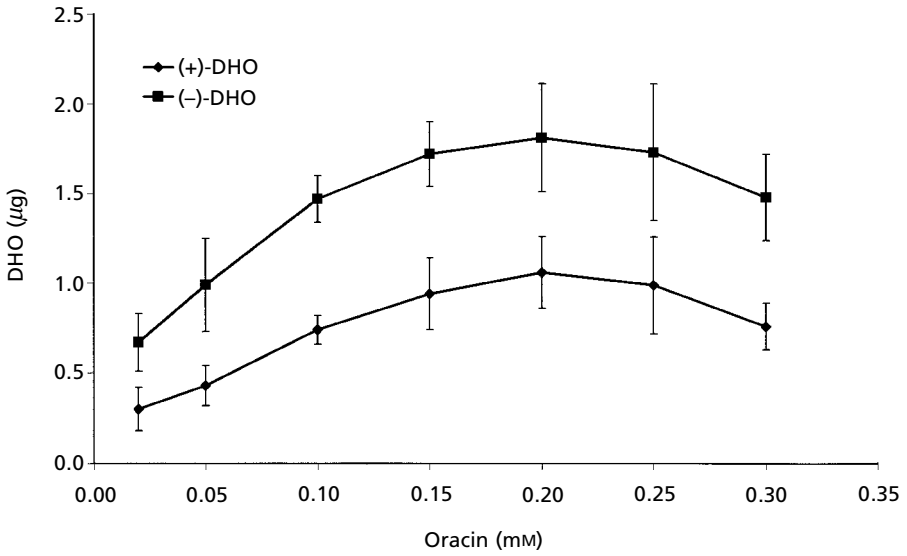

Figure 2 Dependence of bacterial oracin reduction on concentration of oracin. Anaerobic cultivation of caecum bacteria for 24 h. Values represent the average of four determinations (see Materials and Methods for experimental details).

Oracin reduction in vivo

In-vivo study of oracin reduction was performed in male rats. Urine and faeces were collected 48 h after oral administration of oracin (150 mg kg^{-1}). Quantification of DHO in urine and faeces showed that 77% of DHO (386.7 ± 54.3 μg) was excreted in faeces and only 23% (88.3 ± 4.6 μg) in urine. In faeces, the ratio (+)-DHO:(−)-DHO was 60:40 and in urine the ratio was 78:22.

Discussion

Biotransformation of drugs is an integral part of the pharmacokinetic process. Generally, biotransformation consists of a structural change in a drug, aimed at facilitation of its elimination from the organism. The parent substance is transformed into more polar metabolites by attaching or uncovering functional groups. In this way, however, the metabolite can change its biological activity, and its behaviour in an organism can differ from that of the parent substance. Hence the activity of biotransformation enzymes considerably affects toxicological and pharmacological properties of the drug (Testa 1995).

The main route of deactivation of the antitumour drug oracin consists of stereospecific reduction of its carbonyl group to give (+)- and (−)-enantiomers of DHO. Stereospecific reduction of carbonyl group has been reported in other substances as well. The papers published deal with reduction of 17-keto steroids (Winter et al 1984), dehydrocholate and 3-ketoglycyrrhetinate (i.e. their stereospecific reduction to 17β- and 3β-hydroxy derivatives, respectively) (Akao et al 1986). The reduction of some keto steroids catalysed by 3α-hydroxysteroid dehydrogenase/carbonyl reductase (isolated from intestinal bacteria) to 3α-hydroxy steroids is also stereospecific (Opperman et al 1996). This enzyme can also reduce some non-steroidal xenobiotic aldehydes and ketones (e.g. metyrapone (Opperman & Maser 1996)).

Reduction of oracin in subcellular fractions of liver, kidney, lung and heart

Liver is the main organ of biotransformation and is also an important protective barrier against the adverse effects of xenobiotics. A number of enzyme systems are located especially in the endoplasmic reticulum and cytosol of hepatocytes. Some of these enzymes are also found in other organs. Hence, besides liver, other organs can participate in biotransformation processes, though usually to a much lower extent (Krishna & Klotz 1994).

The reduction of oracin, followed in subcellular fractions of kidney, lung and heart, was compared with reduction of oracin in liver. The reduction of oracin in rat liver microsomes is partially performed by CYP1A, but obviously the main enzyme involved is 11β-hydroxysteroid dehydrogenase type 1 (Szotáková et al 2000). In cytosol, carbonyl reductase probably participates in the reduction (Szotáková et al 2000). Some reducing enzymes belonging to the aldo-keto reductase superfamily and the above-mentioned subfamily of cytochrome P450 occur not only in the liver, but also in extrahepatic tissues (Paine et al 1997; Behnia & Boroujerdi 1999; O'Connor et al 1999; Johnson et al 2000; Parkinson 2001). This knowledge led to a presumption that oracin would be reduced not only in liver but in other organs as well. The results obtained confirmed this presumption. Metabolic transformations of oracin take place also in extrahepatic tissues, and important reducing activity towards oracin was observed in extrahepatic tissues. In addition to the activity of oracin reductases, we focused on the stereospecificity of these enzymes.

The activity of microsomal reductases in the heart was very low. In contrast to this, the heart cytosol exhibited the highest specific activity of oracin-reducing enzymes. This reduction could be due to an enzyme belonging to the NADPH-dependent aldo-keto reductases, likewise the reduction of doxorubicin to doxorubicinol in heart cytosol (Behnia & Boroujerdi 1999). Probably, this is a carbonyl reductase reducing a number of carbonyl compounds (inter alia anthracycline antibiotics) and localised in a number of tissues. Hou et al (1994) reported aldo-keto reductases and dihydrodiol dehydrogenases in lung tissue. The microsomal specific activity of oracin reductases in lung tissue distinctly predominated over the cytosolic activity in this organ. It was also the highest microsomal activity among the organs investigated under both aerobic and anaerobic conditions. The resulting value of specific activity was influenced by the difference in protein content between liver and extrahepatic organs (i.e., the highest protein content was found in liver subcellular fractions).

According to expectation, the amount of DHO formed was significantly higher under anaerobic conditions than under aerobic conditions (Table 1) because, in certain cases, oxygen can inhibit the reduction of xenobiotics (Goeptar et al 1995). Under aerobic conditions, a part of DHO is oxidised back to oracin. This reverse transformation was proved by in-vitro experiments in microsomal and cytosolic fractions of liver homogenate and in isolated hepatocytes (Skálová et al 1999) from rat and man (Wsół et al 2000). The presence or absence of oxygen during the in-vitro incubations also affected the stereospecificity of oracin reductases. In fact, in all the organs, in both microsomes and cytosol, the (+)-DHO formation predominated over (−)-DHO formation under all conditions studied.

Reduction of oracin in microsomal and cytosol fractions of intestinal wall

Although the majority of metabolic transformations take place only after absorption of a drug from the gastrointestinal tract, transformation can already start in the intestine or intestinal wall. The metabolism can be influenced by bacterial flora and oxido-reduction and conjugation enzymes localised in epithelial cells of intestinal wall (Ilett et al 1990). Thus the intestinal biotransformation can significantly contribute to the drug biotransformation in-vivo and restrict the bioavailability of perorally administered drugs.

The proportions of enzymatic activity in individual parts of intestinal wall can differ, with the maximum near the pylorus and decreasing distally (Kaminsky & Fasco 1992; Johnson et al 2000). Therefore, the contribution of the intestinal wall enzymes to the oracin biotransformation in rat was evaluated separately in small and large intestine and caecum.

The specific activity of cytosolic reductases was always higher than that of microsomal reductases. Likewise in liver, kidney, lung and heart, the amount of (+)-DHO formed in subcellular fractions of the intestinal wall was always higher than that of (−)-DHO.

Reduction of oracin by intestinal bacteria of rat

The intestinal microflora plays an important role in the metabolism and enterohepatic circulation of drugs (Tancrede 1992). The bacterial flora of the gastrointestinal tract is very complex (Moore & Moore 1995) and any substance administered orally, or entering intestines from bile or blood, becomes a potential substrate for intestinal microorganisms (Chadwick et al 1992; Henderson et al 1997). The ability of microflora to reduce azo and nitro groups of various xenobiotics, mostly dyestuffs (Brown 1981), is long known. Bacterial enzymes mostly exhibit reductive or hydrolytic activity.

A number of papers have been published about the reduction metabolism of drugs by intestinal bacteria. Oxazepam (anxiolytic) is metabolised to desmethyldiazepam (Okamura et al 1996) and zonisamide (anticonvulsant) is reduced by intestinal microflora to 2-sulphamoylacetylphenol (Kitamura et al 1997). Other drugs that are biotransformed by microflora include swertiamarin (El-Sedawy et al 1989), sennosides and sennidines (Hattori et al 1988), sulfinpyrazone and sulindac (Strong et al 1987); 1-phenyl-2-nitropropane is reduced to amfetamine (Mori et al 1990) and 5-fluorocytosine is metabolised to toxic 5-fluorouracil (Harris et al 1986; Malet-Martino et al 1991).

One of our aims was to find out the role of intestinal microflora in oracin deactivation. The cultivation of intestinal bacteria from the lumen of small and large intestines and caecum of rat with oracin confirmed that these bacteria possess the ability to reduce oracin. It was interesting to find that the microbial enzymes participating in the reduction of oracin show an opposite stereospecificity as compared with the enzymes of the organism, forming a higher amount of (−)-DHO.

Under anaerobic cultivation of the bacteria from caecum with oracin at various concentrations, lower amounts of DHO were detected in the samples with lower concentrations of oracin, but this dependence was not linear. These results suggested that increasing the concentration of oracin in the cultivation medium had an inhibitory effect on the growth of some bacteria. In the medium without oracin and with a small amount of intestinal content, a marked growth of bacteria was observed, while in the samples with oracin only a slight turbidity was seen.

Reduction of oracin in rat in vivo

In this in-vivo study, very similar results to our previous data (Wsół et al 1998) were obtained. Shift of the (+)-DHO:(−)-DHO enantiomeric ratio from 90:10 (in liver subcellular fractions) to 60:40 (in faeces) clearly demonstrated the importance of the contribution of extrahepatic metabolism (subcellular fractions of kidney, lung, heart, gut wall and intestinal bacteria) to the total reduction of oracin to DHO.

Conclusions

Oracin is significantly metabolised in extrahepatic organs in-vitro (kidneys, lungs, heart) as well as by bacteria of the intestinal tract of rat. The main metabolite is DHO. Due to the presence of one chiral centre, two enantiomers are formed: (+)-DHO and (−)-DHO. The former was produced to higher extent in subcellular fractions of organs, while the latter was formed in greater amount by the action of intestinal bacteria. The extrahepatic metabolism and the metabolism in the intestinal lumen significantly contribute to the total metabolism of oracin to DHO. It can be presumed that the intestinal flora and the extrahepatic metabolism affect the stereospecificity of reduction of oracin in the rat in-vivo, since the (+)-DHO:(−)-DHO ratio is 90:10 in subcellular fractions of liver (both microsomes and cytosol) and 60:40 in faeces. The results showed that the stereospecificity of enzymes could play an important role in establishing the contribution of individual organs and intestinal bacteria to the reduction of oracin to DHO in-vivo.

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