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Characterization of enzymes responsible for biotransformation of the new antileukotrienic drug quinlukast in rat liver microsomes and in primary cultures of rat hepatocytes

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Abstract

The promising new drug quinlukast, 4-(4-(quinoline-2′-yl-methoxy)phenylsulphanyl)benzoic acid (VÚFB 19363), is under investigation for its anti-inflammatory and anti-asthmatic effects. The main metabolite of quinlukast identified in incubations of rat microsomal fraction, and in primary culture of rat hepatocytes, is quinlukast sulfoxide (M2). Also, several other metabolites of quinlukast were found: two dihydrodiol derivatives (M3, M5) and quinlukast sulfone (M4). This study was conducted to characterize the enzymes involved in quinlukast biotransformation in rat in-vitro. Primary cultures of rat hepatocytes were treated with inducers of different cytochrome P450s (CYPs) for 48 h. Quinlukast (100 μM) was incubated for 24 h in a primary culture of induced or control hepatocytes. The effects of CYP inhibitors, ketoconazole, methylpyrazole, metyrapone and α-naphthoflavone (2, 10, 50 μM), on quinlukast metabolism were tested in induced and control hepatocytes. Significant induction of M2 (6 times), M5 (twice) and M3 (by 50%) formation by dexamethasone and strong concentration-dependent inhibition by ketoconazole indicated that CYP3A participates in formation of these metabolites. CYP1A catalyses formation of metabolite M3 mainly, as β-naphthoflavone induced (10 times) production of M3 and a strong inhibitory effect of α-naphthoflavone on its formation was observed. A significant inhibitory effect of quinlukast (2, 10, 50 μM) on ethoxyresorufin, methoxyresorufin and benzyloxyresorufin *O*-dealkylase activity was observed as well.

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Introduction

Inflammatory reactions involve the release of a wide variety of inflammatory mediators (i.e. prostaglandins, thromboxanes and leukotrienes). Leukotrienes are potent pro-inflammatory mediators involved in the pathophysiology of various inflammatory diseases such as allergic rhinitis, inflammatory bowel disease and asthma (Jain et al 2001). Leukotrienes are responsible for the contraction of human airway and lung vascular smooth muscle. A chemical agent that is effective in blocking the constricting actions of leukotrienes could be used to treat inflammatory processes in the pulmonary system. Anti-leukotriene drugs have become available for the clinical management of asthma and they function either by blocking the interaction of leukotrienes with receptors or by inhibiting leukotriene synthesis (Radhakrishna et al 2002). Recently, cysteinyl leukotriene receptor antagonists (i.e. zafirlukast, montelukast and pranlukast) have been advocated for the treatment of asthma (Chung & Barnes 1998; Vianna & Martin 1998; Weisberg 2000). Zafirlukast, a cysteinyl leukotriene type 1 (Cys-LT_1) receptor antagonist, causes bronchodilation and has anti-inflammatory properties (Savidge et al 1998; Dekhuijzen & Koopmans 2002). Montelukast, a potent and selective antagonist of the Cys-LT_1 receptor, has been shown to block LTD_4-induced bronchoconstriction. It has been approved for the treatment of bronchial asthma (Balani et al 1997; Chiba et al 1997). Quinlukast is a promising new anti-leukotriene drug (4-(4-(quinoline-2′-yl-methoxy)phenylsulphan yl)benzoic acid; VÚFB 19363; Figure 1) (Kuchar et al 2001).

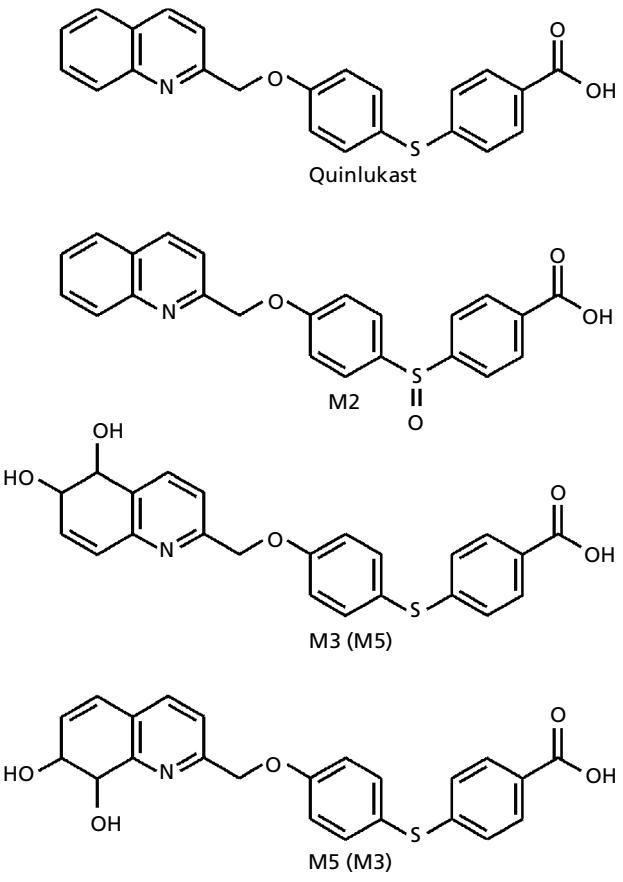

Figure 1 Chemical structure of quinlukast (VÚFB 19363), quinlukast sulfoxide (M2) and its dihydrodiols (metabolites M3 and M5).

Quinlukast was prepared in the frame of synthesis of anti-inflammatory compounds with multiple anti-leukotrienic activity (Kuchar et al 1998). It was characterized (Kuchar et al 2000, 2001) with significant inhibition of LTB_4 biosynthesis and binding to LTD_4 receptors and its good anti-inflammatory activity was manifested by an anti-asthmatic effect in the model of LTD_4-induced bronchospasm in guinea-pigs. The IC50 value (concentration producing 50% inhibition) for quinlukast in inhibiting binding to leukotriene D_4 receptors is comparable with those of montelukast and zafirlukast, while leukotriene B_4 biosynthesis inhibition by quinlukast is much more effective than that of montelukast and zafirlukast (Kuchar et al 2003). The main advantage of quinlukast is its multiple anti-leukotrienic, anti-inflammatory and anti-asthmatic effect.

The main metabolite of quinlukast identified in incubations with microsomal fraction and in incubations with isolated hepatocytes is its sulfoxide (M2). Also, several other metabolites were found, M3, M4 and M5. M4, quinlukast sulfone, is only a minor metabolite. Metabolites M3 and M5 were identified as quinlukast dihydrodiols by means of mass spectrometry (Wsól et al 2003).

This study was conducted to characterize the enzymes involved in quinlukast biotransformation in rat liver microsomal fraction and in primary cultures of rat hepatocytes.

Materials and Methods

Chemicals

Quinlukast (compound VUFB 19363) and quinlukast sulfoxide were obtained from the Research Institute for Pharmacy and Biochemistry (Prague, Czech Republic). Enzymatic tests were performed using NADPH, inhibitors (α-naphthoflavone, ketoconazole, metyrapone, methylpyrazole), and inducers (β-naphthoflavone, phenobarbital, dexamethasone) from Sigma-Aldrich (Prague, Czech Republic). Acetonitrile and methanol, both HPLC grade, were obtained from Riedel-deHaen (Prague, Czech Republic). Collagenase was purchased from Sevapharma (Prague, Czech Republic). The Ham F12 medium, William's E medium, foetal calf serum, antibiotics, insulin and collagen were supplied by Sigma-Aldrich (Prague, Czech Republic). All other chemicals were of the highest purity commercially available.

Animals

Male Wistar rats (10–12 weeks) were obtained from the BioTest (Konárovice, Czech Republic). They were kept on standard rat chow with free access to tap water, in animal quarters under a 12-h light–dark cycle. Rats were cared for and used in accordance with the *Guide for the care and use of laboratory animals* (Protection of Animals against Cruelty Act. No. 246/92 Coll., Czech Republic).

Isolation of microsomal fraction

Livers of 6 rats were homogenised in 0.1 M Na-phosphate buffer, pH 7.4. The microsomal fraction was obtained by fractional ultracentrifugation of the homogenate (Gillette 1971). A re-washing step (followed by second ultracentrifugation) was added at the end of the microsomes preparation procedure. Microsomes were finally resuspended in the homogenisation buffer containing 20% glycerol (v/v) and stored at $-80\,^{\circ}C$. The amount of protein was determined according to the modified method of Lowry with 0.1% SDS (Markwell et al 1978).

Activity of alkoxyresorufin *O*-dealkylases in microsomal fraction

The 7-ethoxyresorufin (EROD), pentoxyresorufin (PROD), methoxyresorufin (MROD) and benzyloxyresorufin (BROD) *O*-dealkylase activity was determined using fluorimetric determination of resorufin (Burke et al 1994) at $37\,^{\circ}C$ with the final concentrations of the substrates 5 μM. The assays were conducted using the Perkin-Elmer luminescence spectrophotometer LS 50B with excitation and emission wavelengths of 530 nm and 585 nm, respectively. The EROD, MROD, PROD and BROD activity (corresponding to CYP1A1/2, CYP1A2/1, CYP2B and

CYP2B/3A activity, respectively) was also measured in the presence of 2, 10 or 50 μM quinlukast. All activity was calculated using the standard amount–addition technique (Burke et al 1985, 1994).

Incubation of microsomal fraction with quinlukast

The liver microsomal suspension (50 μL, 0.5 mg of protein) was incubated in Eppendorf microtubes with 200 μM quinlukast as a substrate and 0.5 mM NADPH in a total buffer volume of 0.3 mL. Selective CYP inhibitors α-naphthoflavone (CYP1A), ketoconazole (CYP3A), metyrapone (CYP2B), methylpyrazole (CYP2E1) were used in final concentrations of 2–50 μM. Flavin-containing monooxygenase (FMO) activity was selectively inactivated by pre-incubation of rat liver microsomal suspension at 45 °C for 5 min. Incubations were performed for 30 min at 37 °C. All incubations were terminated by cooling to 0 °C and adding 10 μL 1 M HCl. Samples were extracted twice with two volumes of ethyl acetate–n-heptane (1:3, v/v), and combined extracts were evaporated to dryness. The dry samples were dissolved in 5 mM phosphate buffer (pH 7.0) and prepared for HPLC injection.

Isolation of hepatocytes

Hepatocytes were obtained by a two-step collagenase method (Berry et al 1991). In the first step the liver was washed by 150–200 mL of solution without calcium with the aim to remove the rest of the blood and make the cell–cell junction weaker. In the second step hepatocytes were released by the action of collagenase (50 mg/100 mL) in perfusion solution. The second perfusion lasted 5–6 min (re-circulation system). Isolated hepatocytes were rewashed three times and mixed together with culture medium. The culture medium consisted of a 1:1 mixture of Ham F12 and Williams' E, supplemented as described earlier (Isom & Georgoff 1984; Maurel 1996). The viability of cells measured by Trypan blue staining according to Sigma protocol was 85–90%. Three million viable cells in 3 mL of culture medium were placed into 60-mm plastic dishes pre-coated with collagen. The foetal calf serum was added in culture medium (5%) to favour cell attachment during the first 4 h after plating. Then the medium was changed for fresh medium without serum. The cultures were maintained at 37 °C in a humid atmosphere of air and 5% CO_2.

Induction of hepatocytes

Stock solutions of inducers dissolved in dimethyl sulfoxide (DMSO) were added to fresh medium. The final concentration of DMSO in medium was 0.1%. Primary cultures of rat hepatocytes were treated with dexamethasone (10 μM) (CYP3A), phenobarbital (1 mM) (CYP2B), ethanol (0.2 M) (CYP2E1) and β-naphthoflavone (10 μM) (CYP1A) for 48 h. The medium of control hepatocytes contained 0.1% of DMSO.

Incubation of hepatocytes primary culture with quinlukast

The specific inhibitors in various concentrations (0, 2, 10, 50 μM) were added into control hepatocyte cultures or hepatocyte cultures treated with corresponding inducer at the same time as quinlukast (100 μM). Ketoconazole was used in dexamethasone-treated hepatocytes, metyrapone in phenobarbital-treated hepatocytes, methylpyrazole in ethanol-treated hepatocytes and α-naphthoflavone in β-naphthaflavone-treated hepatocytes. In control hepatocytes all inhibitors were tested. Incubation of quinlukast in control or induced hepatocytes with or without inhibitors lasted for 24 h. At the end of the incubation, samples of medium (0.5 mL) were collected. Samples were stored frozen at −80 °C before analyses.

Cytotoxicity test

The cytotoxic effect of quinlukast (0.1 mM), dexamethasone (10 μM), phenobarbital (1 mM), ethanol (0.2 M) and β-naphthoflavone (10 μM) on rat hepatocytes was assessed after 48 h exposure, using the MTT (dimethylthiazol diphenyl tetrazolium bromide) test as described by Denizat & Lang (1986). The absorbance of the product, formazan, in cells treated with quinlukast was compared with that in control cells exposed to medium with 0.1% DMSO alone.

Solid-phase extraction

Solid-phase extraction (SPE) of the hepatocyte samples was made on Sep-Pak Light tC18 cartridges (145 mg sorbent; Waters, Prague, Czech Republic). The cartridges were conditioned with methanol (2 mL) and washing solution of 10 mM phosphate buffer (2 mL, pH 7.0) before applying the samples. Hepatocyte samples (0.5 mL) prepared as described above were manually loaded onto the cartridges, which were purged with washing solution (4.5 mL). Finally, all SPE cartridges were eluted with 1 mL methanol. The eluting solvents were evaporated, reconstituted in 120 μL solution consisting of 5 mM phosphate buffer (pH 7.0) in acetonitrile–H_2O (40:60 v/v) and prepared for HPLC injection.

HPLC assay for quinlukast and its metabolites

Quinlukast and its metabolites were measured by an HPLC system from Shimadzu (Prague, Czech Republic) consisting of LC-10 AD_{VP} gradient pump, SIL-10 AD_{VP} autoinjector, FCV-10 AL_{VP} solvent mixer, CTO-10 AC_{VP} column oven, SCL-10 A_{VP} system controller and SPD-M10 A_{VP} UV-Vis photodiode array detector with detection set at 220–300 nm range (285 nm for metabolites). Data from these chromatographic runs were processed using the Chromatography Laboratory Automated Software System Class VP (version 6.12) from Shimadzu (Prague, Czech Republic) on a 586/100 PC from AutoCont (Hradec Králové, Czech Republic). An assay was performed on a Purospher RP18e analytical column (5 μm, 125 × 3 mm; Merck, Prague, Czech Republic)

equipped with a Hypersil BDS C18 guard column (5 μm, 4 × 4 mm, Agilent, Prague, Czech Republic). The HPLC method involved the following gradient system using 8 mM H_3PO_4 in H_2O–methanol–acetonitrile (70:16.5:13.5, v/v/v) as mobile phase A and 8 mM H_3PO_4 in H_2O–methanol–acetonitrile (10:49.5:40.5, v/v/v) as mobile phase B. The flow rate was set at 0.7 mL min^{-1}. From 0 to 13 min, the ratio of mobile phase A to B was linearly changed from 73:27 to 51:49. Mobile phase B was then increased to 80% at 13.5 min. Mobile phase B was held at 80% for 6.5 min, after which it was reverted back to 27% at 20.5 min. The equilibration time was 15.5 min. All chromatographic runs were performed at 25 °C.

Blank medium spiked with quinlukast sulfoxide was used for calibration. Weighed linear regression analysis was done by plotting peak area versus analyte concentration (0.08–8.00 μmol L^{-1}). Regression line parameters were used to calculate concentrations of other metabolites in all biological samples. The limit of reliable quantification (CV ≤ 20%) was taken as 80 nmol L^{-1} for all three metabolites. Intra-day variability was assessed by quadruplicate analyses of incubated samples. The CV values did not exceed 4.9% (quinlukast sulfoxide, M2), 5.1% (M3) and 7.2% (M5), respectively. Absolute recovery of M2 from medium was 84.7 ± 2.6% at the 2.4 μmol L^{-1} level.

Statistical analysis

In the study of quinlukast biotransformation in rat liver microsomes and in primary cultures of rat hepatocytes, the one-way analysis of variance was performed for comparison of induction or inhibition effect; data from 4–6 individual incubations (n = 4–6) were compared.

Results

Metabolism of quinlukast in rat liver microsomes

Rat liver microsomal fraction was incubated with quinlukast and influence of CYP and FMO inhibition on quinlukast biotransformation was studied. Results are presented in Table 1.

The marked thermal lability of the hepatic FMO in conditions above 35 °C in the absence of NADPH (45 °C for 5 min) was used to define the contribution of FMO in the sulfoxidation pathway. Inactivation of FMO had no effect on quinlukast sulfoxidation, but slightly lowered formation of M3 and M5 ($P < 0.05$).

Selective inhibitor of CYP 3A ketoconazole (2–50 μM) caused significant dose-dependent inhibition of all metabolites (M2, M3 and M5) formation. α-Naphthoflavone, a CYP1A1/2 inhibitor, in concentrations of 2 and 10 μM enhanced formation of M2 and in all concentrations inhibited M3 formation. α-Naphthoflavone in higher concentrations (10 and 50 μM) also lowered formation of M5. Other CYP inhibitors used – metyrapone and methylpyrazole – had no effect on formation of M2, but at the concentration of 50 μM methylpyrazole lowered the production of M3 and M5 ($P < 0.01$) and metyrapone lowered M5 only ($P < 0.05$).

Cytotoxicity assay

Quinlukast (0.1 mM), dexamethasone (10 μM), phenobarbital (1 mM), ethanol (0.2 M) and β-naphthoflavone (10 μM) were shown to be non-cytotoxic in primary cultures of rat hepatocytes.

Table 1 Effect of CYP and flavin-containing monooxygenase (FMO) inhibition on biotransformation of quinlukast in rat liver microsomes.

		Specific activity (pmol/30 min/mg protein)		
	Concn of inhibitor (μM)	M2	M3	M5
Control	0	1533.6 ± 98.4	257.1 ± 15.9	296.8 ± 12.0
α-Naphthoflavone	2	1860.1 ± 104.1*	215.8 ± 10.2*	283.0 ± 5.9
	10	2117.9 ± 189.0**	196.0 ± 23.6*	238.5 ± 21.9*
	50	1433.4 ± 116.1	157.2 ± 16.5***	173.4 ± 8.4***
Ketoconazole	2	1088.0 ± 101.6**	243.7 ± 16.3	277.7 ± 14.6
	10	784.0 ± 74.0***	178.2 ± 23.7**	218.7 ± 32.1**
	50	344.0 ± 32.8***	76.0 ± 7.5***	96.7 ± 6.8***
Metyrapone	2	1902.7 ± 76.9	222.9 ± 15.6	282.2 ± 28.3
	10	2273.5 ± 101.0	197.9 ± 26.5	241.3 ± 28.7
	50	1988.9 ± 216.4	204.0 ± 12.1	255.6 ± 5.6*
Methylpyrazole	2	1813.9 ± 170.2	231.6 ± 4.6	271.8 ± 6.2
	10	1912.6 ± 89.2	221.5 ± 19.2	259.6 ± 17.7
	50	1941.3 ± 208.3	189.8 ± 8.6**	212.4 ± 10.4**
Heat	0	1495.3 ± 130.2	202.3 ± 5.7*	256.0 ± 13.2*

Concentration of quinlukast was 200 μM, concentrations of inhibitors ketoconazole, α-naphthoflavone, metyrapone and methylpyrazole were 2, 10 or 50 μM. FMO was inactivated at 45 °C for 5 min. Values are given as mean ± s.d. from 4 experiments. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, compared with the control.

Metabolism of quinlukast in rat hepatocytes

Primary cultures of rat hepatocytes were treated with inducers of different CYPs (dexamethasone, phenobarbital, ethanol, β-naphthoflavone) for 48 h. Quinlukast (100 μM) was incubated for 24 h in primary culture of induced or control hepatocytes. The effects of CYP inhibitors, ketoconazole, methylpyrazole, metyrapone and α-naphthoflavone (2, 10, 50 μM) on quinlukast metabolism were also tested both in induced and control hepatocytes. The medium samples (0.5 mL) were HPLC analysed and quinlukast metabolites were quantified. Results are shown in Figures 2–5. Significant ($P < 0.001$) induction of M2 formation (6 times) by dexamethasone (10 μM) and strong concentration-dependent inhibition by ketoconazole was observed. Neither β-naphthoflavone, nor phenobarbital nor ethanol had any effect on quinlukast sulfoxidation (data not shown here). In contrast to the microsomal fraction, a similar effect of α-naphthoflavone on M2 formation was observed.

Dexamethasone also induced formation of metabolite M5 (twice) and M3 (by 50%) ($P < 0.001$), and ketoconazole strongly inhibited these metabolites. CYP1A inducer, β-naphthoflavone, induced metabolite M3 by a factor of 10 and α-naphthoflavone was observed to have a strong inhibitory effect on its formation.

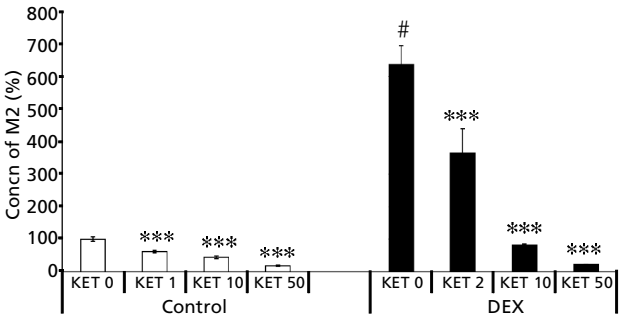

Figure 2 Effect of ketoconazole (KET) on sulfoxidation of quinlukast in primary cultures of rat hepatocytes treated for 48 h with CYP inducer dexamethasone (DEX, 10 μM) and in control hepatocytes. Concentration of substrate was 100 μM, concentration of inhibitor KET was 0, 2, 10 or 50 μM. Values are given as mean ± s.d. from 4 experiments. See Materials and Methods section for experimental details. #$P < 0.001$ compared with the control hepatocytes (non-induced, KET 0); ***$P < 0.001$ compared with the non-inhibited hepatocytes (KET 0).

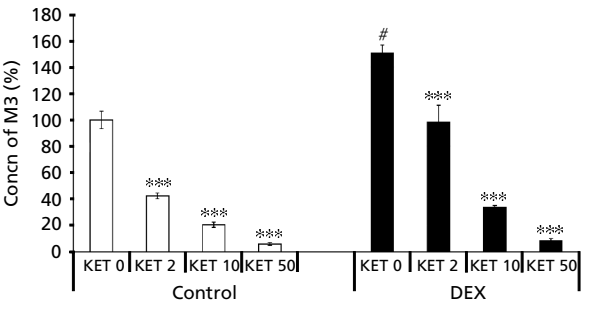

Figure 4 Effect of ketoconazole (KET) on formation of M3 in primary cultures of rat hepatocytes treated for 48 h with CYP inducer dexamethasone (DEX, 10 μM) and in control hepatocytes. Concentration of quinlukast was 100 μM, concentration of inhibitor KET was 0, 2, 10 or 50 μM. Values are given as mean ± s.d. from 4 experiments. See Materials and Methods section for experimental details. #$P < 0.001$ compared with the control hepatocytes (non-induced, KET 0); ***$P < 0.001$ compared with the non-inhibited hepatocytes (KET 0).

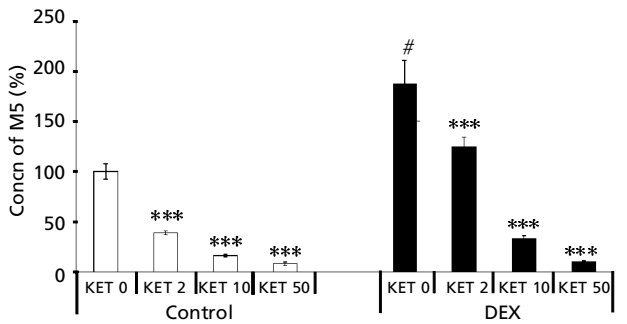

Figure 3 Effect of ketoconazole (KET) on formation of M5 in primary cultures of rat hepatocytes treated for 48 h with CYP inducer dexamethasone (DEX, 10 μM) and in control hepatocytes. Concentration of quinlukast was 100 μM, concentration of inhibitor KET was 0, 2, 10 or 50 μM. Values are given as mean ± s.d. from 4 experiments. See Materials and Methods section for experimental details. #$P < 0.001$ compared with the control hepatocytes (non-induced, KET 0); ***$P < 0.001$ compared with the non-inhibited hepatocytes (KET 0).

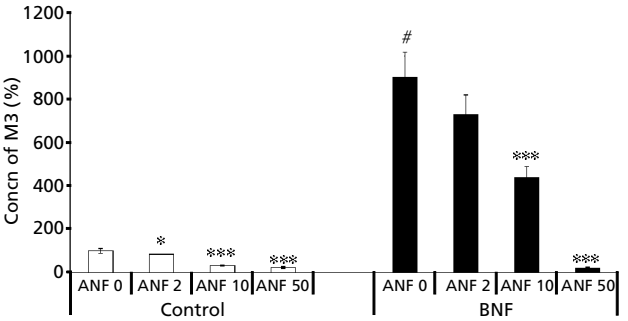

Figure 5 Effect of α-naphthoflavone (ANF) on formation of M3 in primary cultures of rat hepatocytes treated for 48 h with CYP inducer β-naphthoflavone (BNF, 10 μM) and in control hepatocytes. Concentration of quinlukast was 100 μM, concentration of inhibitor ANF was 0, 2, 10 or 50 μM. Values are given as mean ± s.d. from 4 experiments. See Materials and Methods section for experimental details. #$P < 0.001$ compared with the control hepatocytes (non-induced, ANF 0); *$P < 0.05$, ***$P < 0.001$ compared with the non-inhibited hepatocytes (ANF 0).

Effect of quinlukast on CYP activity in rat liver microsomes

The activity of individual isoenzymes of cytochrome P450 towards their selective substrates was measured spectrofluorimetrically in microsomes. Results are shown in Figure 6. MROD, EROD, PROD and BROD activity of rat liver microsomes was compared with the activity measured in the presence of quinlukast (2, 10, 50 μM). Quinlukast, even in low concentrations, significantly ($P < 0.001$) lowered the MROD, EROD and BROD activity.

Discussion

The aim of our study was to characterize the enzymes involved in quinlukast biotransformation in rat liver microsomal fraction and in primary cultures of rat hepatocytes. Quinlukast belongs to the cysteinyl leukotriene receptor antagonists, like zafirlukast, montelukast, pranlukast and verlukast.

The metabolism of these drugs is well described (Grossman et al 1993; Balani et al 1997; Chiba et al 1997; Savidge et al 1998; Ravasi et al 2002). It has been shown that sulfur-containing functional groups are generally subject to CYP or FMO-catalysed oxidation. The contribution of FMO was investigated in the sulfoxidation of montelukast. Although sulfur-containing drugs are generally excellent substrates for FMO, carboxylic acids containing a sulfide group on the terminal carbon have not shown detectable substrate activity (Ziegler 1990, 1993). The presence of a carboxylic acid on the same side-chain structure as the sulfide may similarly prevent montelukast from being a favourable substrate for FMO in human liver microsomes. It has been reported that among the metabolic pathways for montelukast, CYP was found to catalyse exclusively all montelukast oxidations (Chiba et al 1997).

The same phenomenon was observed in the case of quinlukast, as no decrease of its sulfoxidation after inhibition of FMO was found. On the other hand, FMO may partially catalyse formation of M3 and M5, where significant ($P < 0.05$) decrease after FMO inhibition was found.

Biotransformation of quinlukast was further studied from the aspect of the respective contribution of individual CYP isoforms to the process. As CYP1A, CYP2B, CYP2E1 and CYP3A are known to account for a major part of the CYP activity in the rat (Nelson et al 1993; Testa 1995), specific substrates, inducers and inhibitors of these CYP isoforms were chosen for our experiments.

β-Naphthoflavone is a well-known inducer of CYP1A, while phenobarbital and ethanol are often used for induction of CYP2B and CYP2E1, respectively. Dexamethasone has been reported to be a very potent inducer of CYP3A isoforms in rat hepatocytes but was less potent in human hepatocytes (Lu & Li 2001). In rat liver microsomal fraction, α-naphthoflavone was used as an inhibitor of CYP1A. To inhibit CYP2B isoforms, metyrapone, a relatively specific inhibitor in the rat, was used. Methylpyrazole was used to inhibit cytochrome CYP2E1. Ketoconazole was used to confirm participation of CYP3A in quinlukast biotransformation.

Dexamethasone pre-treatment of hepatocytes caused significant induction of M2 (6 times), M5 (twice) and M3 (by 50%). Strong concentration-dependent inhibition of formation of these metabolites by ketoconazole was observed. On the other hand, CYP1A1/2 inhibitor α-naphthoflavone enhanced formation of M2 in rat liver microsomes (Table 1). This observation can be explained by the fact, that flavones, such as α-naphthoflavone, are in-vitro activators of CYP3A (Parkinson 2001). With respect to all results mentioned above, significant participation of CYP3A in the production of metabolite M2 and to a lesser extent of metabolites M5 and M3 is proposed.

In primary culture of rat hepatocytes β-naphthoflavone induced production of M3 by a factor of 10. A strong inhibitory effect of α-naphthoflavone on M3 formation was observed. Formation of other metabolites was not affected. In rat liver microsomes, α-naphthoflavone also significantly inhibited M3 formation, even at low concentrations (2 μM, $P < 0.05$; 10 μM, $P < 0.05$; 50 μM, $P < 0.001$), but formation of M5 was also inhibited (10 μM, $P < 0.05$; 50 μM, $P < 0.001$). These results showed that CYP1A mainly catalyses formation of metabolite M3, but participation of CYP1A in M5 formation cannot be excluded.

Similarly to montelukast (Chiba et al 1997), sulfoxidation of quinlukast (formation of M2) is catalysed by CYP3A and other CYPs studied (CYP1A, CYP2B and CYP2E1) had little or no effect on its formation. CYP1A and CYP3A (to a different extent) participate in production of quinlukast metabolites M3 and M5, which were identified as quinlukast dihydrodiols. Involvement of CYP1A1 in the formation of dihydrodiol of verlukast was reported (Grossman et al 1993; Nicoll-Griffith et al 1993). Similarly to our results, β-naphthoflavone enhanced formation of verlukast dihydrodiol and α-naphthoflavone caused inhibition. On the other hand, CYP3A probably takes part in quinlukast biotransformation to M3 and M5 metabolites as well, but not in the case

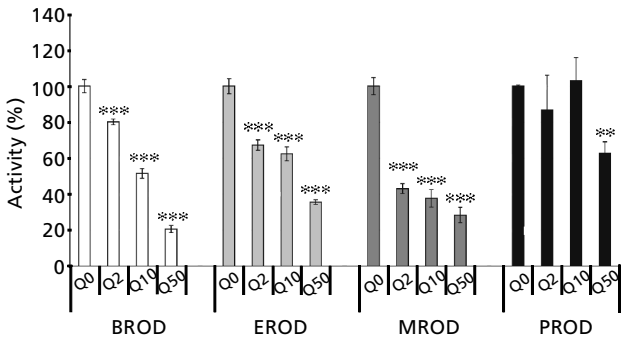

Figure 6 Effect of quinlukast (Q) on CYP activity (BROD, EROD, MROD and PROD) in rat microsomes. Concentration of quinlukast was 0, 2, 10 or 50 μM. Values are given as mean ± s.d. from 6 experiments. $^{**}P < 0.01$, $^{***}P < 0.001$ compared with the control (Q 0).

of verlukast (Grossman et al 1993; Nicoll-Griffith et al 1993).

Metabolism of quinlukast is mediated mainly by CYP isoforms (CYP1A, CYP3A) in rat. As ethoxy-, methoxy- and benzyloxyresorufin are considered to be relatively specific substrates for CYP1A2/1, CYP1A1/2 and CYP2B/3A, respectively (Burke et al 1994), co-administration of quinlukast with these model substrates was investigated. EROD, MROD and BROD activity in the presence of quinlukast was significantly lower, even at a low concentration of quinlukast (2 μM, Figure 6). This phenomenon can probably be explained as competition between two substrates, but another mechanism cannot be excluded. However, when some other drug, which is a substrate of CYP1A or CYP3A, is administered simultaneously with quinlukast, drug–drug interactions can occur.

Conclusions

This biotransformation study of the new antileukotrienic drug quinlukast in rat liver microsomes and in primary cultures of rat hepatocytes indicates that oxidative metabolism of quinlukast is mediated mainly by CYP isoforms. CYP3A catalyses quinlukast sulfoxidation to metabolite M2, formation of metabolite M5 and, partially, metabolite M3. CYP1A catalyses formation of metabolite M3 and, partially, also metabolite M5.

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