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An In Vitro Study on Methotrexate Hydroxylation in Rat and Human Liver

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Summary

Methotrexate (MTX) was investigated for possible effect on the metabolism of ethoxyresorufin, pentoxyresorufin and ethoxycoumarin, the model substrates of cytochrome P450. The investigation was carried out in liver microsomes of rats pretreated with classical inducers of cytochrome P450 as well as in microsomes of two human livers. Furthermore, we measured the conversion of MTX (100 μM) to its main metabolite, 7-hydroxymethotrexate (7-OHMTX), in microsomes and cytosolic fractions of rat and human livers. The inhibition of 7-OHMTX formation by menadion (inhibitor of aldehyde oxidase) and allopurinol (inhibitor of xanthine oxidase) was studied in the cytosol of rat and human livers. In both species, MTX in the concentration range 0.5–500 μM exerted no inhibitory effect on enzymatic activities associated with cytochrome P450. Moreover, we did not observe any measurable formation of 7-OHMTX in liver microsomes. MTX was metabolized at a similar rate in the cytosol of rat and human liver. Allopurinol (100 μM) reduced the rate of MTX hydroxylation by 31.5 % in the cytosol of human livers but had no effect in the rat. Menadion (100 μM) decreased the rate of 7-OHMTX formation in the cytosol of human and rat liver by 69 % and 94 %, respectively. Our results confirmed that MTX is oxidized by a soluble enzymatic system in both the rat and human liver. In human tissues, both aldehyde oxidase and xanthine oxidase may play an important role in the metabolism of MTX. Depression of cytochrome P450 and related enzymatic activities observed *in vivo* cannot be explained by a direct inhibitory action of MTX on cytochrome P450.

Key words

Methotrexate – Cytochrome P450 – 7-hydroxymethotrexate – Menadion – Allopurinol – *In vitro* inhibition – Rat and human liver

Introduction

Methotrexate (MTX), a folic acid antagonist, is one of the most commonly used cytostatics. MTX is used in many different malignant diseases, has a large dose-response range and can be administered by various routes.

Although renal excretion is the major route of MTX elimination, a significant part of the dose is secreted into bile by an active process. The liver is the principal site of MTX conversion to 7-hydroxymethotrexate (7-OHMTX), the major extracellular metabolite. In man, the steady-state serum concentrations of 7-OHMTX were found to be proportional to doses from 0.5 to 33.6 g/m^2 of MTX, given in a 24 h i.v. infusion. Moreover, the serum concentration of 7-OHMTX was found to exceed that of MTX several hours after the end of the infusion. The conversion of MTX to 7-OHMTX has been demonstrated to occur in patients on either high or low dose regimens. Furthermore, there is a great interindividual variability both in the extent and time course of the metabolite formation (Borsi *et al.* 1990). In preclinical studies (Fabre *et al.* 1983, Goldman *et al.* 1985), 7-OHMTX has been demonstrated to interact with the pharmacokinetics and the pharmacodynamics of MTX by affecting its intracellular entry and polyglutamation. 7-OHMTX exerts much lower toxicity on isolated tumour cells *in vitro* but its low solubility in aqueous solutions is the cause of its high potential nephrotoxicity following intermediate- or high-dose MTX therapy (Bremnes *et al.* 1991).

The enzymology of 7-OHMTX formation in man does not appear to have been studied sufficiently. In rabbits, MTX hydroxylation proceeds rapidly in several tissues by the cytosolic enzyme aldehyde oxidase (ALO) (EC 1.2.3.1.). 7-OHMTX formation in man and rat is slower by more than one order of magnitude and the enzyme(s) responsible are not certain (Bremnes *et al.* 1991, Sasaki *et al.* 1983). Xanthine oxidase (XO) (EC 1.2.3.2.), a structurally similar enzyme to ALO, is thought to play a more significant role than ALO in the metabolism of heterocyclic compounds in human tissues.

There is convincing evidence in the literature of the *in vivo* interaction of MTX with cytochrome P450 (EC 1.14.14.1). The administration of a high dose of MTX (75 mg/kg) to rats resulted in decreased metabolism of aminopyrine and markedly reduced both the content of cytochrome P450 in the liver and the activity in the cytochrome P450-associated monooxygenase system (Guitton *et al.* 1994). Lukienko *et al.* (1993) described a depression of monooxygenase activities in liver microsomes, a drop of the cytochrome P450 concentration as well as a decrease of the inducing effect of phenobarbital on liver metabolic activity in rats after repeated low-dose MTX (0.25 mg/kg) administration. Theophylline clearance decreased by 19 % in patients after low-dose MTX therapy in double blind, placebo-controlled trial (Glynn-Barnhart *et al.* 1991).

The purpose of this work was to study the mechanism of the interaction between MTX and cytochrome P450 from two main aspects:

– is there any MTX hydroxylating activity in the microsomal fraction of rat and human liver?

– does MTX inhibit cytochrome P450-associated monooxygenase activities (dealkylation of ethoxyresorufin, pentoxyresorufin and ethoxycoumarin) in the hepatic microsomes of rat and human origin?

Furthermore, we investigated 7-OHMTX formation in the cytosolic fraction of the rat and human liver. The effect of menadion (an inhibitor of ALO) and allopurinol (an inhibitor of XO) on the cytosolic MTX-hydroxylase was also assessed in both species.

Material and Methods

Pretreatment of animals

Male Wistar rats (155–230 g) were given sodium phenobarbiton (PB, 80 mg/kg in saline i.p. for four days) or β-naphtoflavone (BNF, 40 mg/kg in corn oil i.p. for three days) or dexamethasone (DEX, 80 mg/kg in corn oil i.p. for three days). Control rats were given appropriate vehicle (saline or corn oil i.p.). Each pretreatment was given in a single dose per day at 08:00–09:00 h. All rats had free access to standard chow and water and were kept at a constant temperature (25 °C). They were fasted for 12 h before experiments.

Preparation of liver microsomes

Twenty-four hours after the last pretreatment, rats were anaesthetized with urethane (1.5 g/kg), the portal vein was cannulated and the liver was perfused to rinse away blood (ice cold saline, 2 units/ml heparin). The microsomal fraction of the liver was prepared as follows: liver samples were homogenized in 4 ml/g tissue homogenizing medium (0.154 M KCl, 50 mM Tris, pH 7.4) with a Potter-Elvehjem glass homogenizer (6 strokes at 1200 rpm). The post-mitochondrial supernatant fraction was prepared by centrifugation at 10 000*xg* for 20 min at 4 °C. The microsomal fraction was prepared by ultracentrifugation (Beckman L 5.50 ultracentrifuge) of post-mitochondrial supernatant at 105 000*xg* for 1 h at 4 °C. The microsomal pellet was resuspended in homogenizing medium (1 ml of the suspension contained microsomes from 0.5 g of rat liver) and stored at −80 °C until used (not longer than 2 months).

Samples of human liver were obtained from two donors for kidney transplantation who died accidentally. The donors were free of any metabolic disease and they received no medication which is known to influence the activity of cytochrome P450. The samples were removed within 30 min after death, cooled at 4 °C and stored at −80 °C within 1 h until used. Microsomes were prepared as described for the rat liver.

Protein determination

The protein concentration in microsomal suspensions and in 105 000*xg* supernatant was determined using the bicinchoninic acid reagent (Pierce Rockford, IL) (Smith *et al.* 1985). Bovine serum albumin (Fraction V, Sigma) was used for the calibration.

Cytochrome P450 determination

The content of cytochrome P450 was determined in liver microsomes by the method of Omura and Sato (1964) using a dual-wavelength spectrophotometr Shimadzu.

Determination of MTX and 7-OHMTX

Concentrations of MTX and 7-OHMTX were assayed by HPLC. An equal volume of 0.4 M trichloracetic acid (TCA) to 100 μl of a cytosolic fraction of the liver was added to precipitate proteins. The sample was vortexed for 5 s and centrifuged at 1 100*xg* for 10 min. 20 μl (MTX assay) or 50 μl (7-OHMTX assay) of the supernatant were directly injected onto the HPLC system, which consisted of the following components: LC10AD pump and SPD10A spectrophotometric detector (Shimadzu, Kyoto, Japan); model 7125 injector (Rheodyne, Cotati, California); an 150 x 3 mm analytical column and a 30 x 3 mm precolumn packed with Separon S6XC18 (7 μm,

Tessek, Prague, Czech Republic). The mobile phase consisted of 0.1 mol/l sodium acetate containing 13 % acetonitrile (pH 4.4 adjusted by glacial acetic acid). The flow rate was 0.6 ml/min and the effluent was monitored at a wavelength of 303 nm. The retention times were 7.5 min (MTX) and 9.2 min (7-OHMTX). MTX and 7-OHMTX concentrations were estimated by comparing the peak area with standard curves prepared daily, using cold blank cytosol spiked with MTX (10–150 μM) or 7-OHMTX (0.1–20 μM). 7-OHMTX (the HPLC purity 99 %) was prepared using rabbit liver aldehyde oxidase purified to the ammonium sulfate step (Sasaki *et al.* 1983). The mean recovery of MTX and 7-OHMTX from hepatic cytosol was 86 % and 80 %, respectively, over the whole concentration range.

MTX incubation in microsomal suspensions

Microsomal proteins, corresponding to 0.5–2 nmol cytochrome P450, were incubated in a final volume of 1 ml of 50 mM Tris buffer, pH 7.4, 5 mM $MgSO_4$, 50 mM KCl, 1 mM NADP and 5 mM isocitrate. MTX was added to a final concentration of 100 μM. The reaction was started by addition of isocitrate dehydrogenase (to a final concentration 0.5 U/ml). After 30 min at 37 °C, 1 ml of 0.8 M TCA was added to precipitate proteins. Alternatively 1 mM NADPH was used instead of NADPH regenerating system.

MTX incubation in hepatic cytosol

MTX (100 μM) was incubated in the cytosolic fraction of rat and human liver (pH = 7.4) at 37 °C and 100 μl aliquots of the reaction mixture were transferred into 100 μl of 0.8 M TCA at 10, 20 and 30 min after MTX addition to the reaction mixture. In inhibition studies, menadion (100 μM) or allopurinol (100 μM) were preincubated with the cytosol for 15 min before the addition of MTX.

Monooxygenase activities

The activity of ethoxyresorufin deethylase (EROD) and pentoxyresorufin depentylase (PROD) was measured fluorimetrically using a Perkin-Elmer LS 50B Luminescence Spectrometer as reported elsewhere (Burke *et al.* 1985). The assay was carried out at 37 °C using a 1 cm path cell with excitation and emission wavelenghts of 530 nm and 585 nm. The reaction mixture contained:

Buffer	Tris HCl, 0.1 M, pH 7.4
Protein level	200–400 mg/ml for control rats 5–50 μg/ml for inducer-treated rats (depending on the type of inducer)
Substrate	5 μM (in dimethylsulfoxide)
NADPH	1 mM
Cofactor	$MgCl_2$, 5 mM

Linearity with respect to the time and protein content was controlled before starting the inhibition study. The reaction mixture containing microsomal protein, substrate, cofactor and buffer, was equilibrated for 5 min at 37 °C and the reaction was started with NADPH. The reaction rate was measured by the increasing fluorescence and the extent of the increase was calibrated by the addition of a 10 μl aliquot of resorufin (10 μM in dimethylsulfoxide) into 1 ml of the reaction mixture.

The activity of ethoxycoumarin deethylase (ECDE) was measured by a similar method and the following composition of the reaction mixture was employed:

Buffer	Tris HCl, 0.1 M, pH 7.4
Protein level	100–300 μg/ml for controls 20–50 μg/ml for inducer-treated rats
Substrate	250 μM (in dimethylsulfoxide)
Cofactor	$MgCl_2$, 5 mM
NADPH	1 mM

The increase of fluorescence was monitored at excitation and emission wavelengths of 370 nm and 452 nm and calibrated by the addition of 10 μl 7-OH coumarin (200 μl, dissolved in ethanol and diluted with water, ethanol 0.1 % final in the reaction mixture). Inhibition studies: MTX (0.5–500 μM) was preincubated for 5 min at 37 °C in the reaction mixture for monooxygenase activity measurements prior to the addition of the substrate and NADPH. Metyrapone and quinidine (0.5–500 μM), model inhibitors of cytochrome P450, were used as positive controls.

Results

After repeated administration of PB, the liver weight was increased by 25 % in the group of inducer-treated rats as compared to the controls (pretreated with 0.9 % NaCl). There was no difference in liver weight between control group (pretreated with corn oil) and groups pretreated with either DEX or BNF (Table 1). Surprisingly, the liver weight was higher in the control group 1 (corn oil) than in the control group 2 (saline).

Nevertheless, the livers of oil-pretreated rats showed normal macroscopic appearance and there was no difference in the enzyme activities between the two groups (Table 1 and 2). All three inducers elevated the content of cytochrome P450 by approximately 200 % as compared to the appropriate vehicles (Table 1). The activity of PROD was extensively (30-fold) and selectively enhanced by PB. On the contrary, neither BNF nor DEX induced PROD activity. The rank order for the degree of EROD induction was: BNF > DEX > PB. The extent of ECDE induction was modest, the highest activity was found after BNF pretreatment (Table 2). Cytochrome P450

concentration, EROD and ECDE activities in microsomes prepared from the two samples of the human liver are summarized in Table 3. The constitutional activity of PROD in human microsomes was too low to be measured.

Table 1. Comparison of body and liver weights and cytochrome P450 content following repeated administration of phenobarbital (PB), dexamethasone (DEX) and β-naphtoflavone (BNF) or appropriate vehicles to male Wistar rats (N=3 in each group).

Group	Body weight (g)	Liver weight (g)	Liver/Body weight (%)	Cytochrome P450+ (nmol/mg)
Control				
Corn oil	167±6.9	9.7±1.1	5.8±0.71	0.63±0.16
0.9 % NaCl	197±17.1	7.6±1.4	4.0±0.32	0.51±0.16
Induced				
PB	210±8.4	11.5±1.1*	5.2±0.29*	2.0±0.37**
DEX	167±4.7	10.1±0.5	6.1±0.16	2.4±0.64**
BNF	180±7.1	11.5±2.1	6.5±1.46	2.2±0.76**

*Data are means±S.D. Significant difference from control (Mann-Whitney test, * $p<0.05$, ** $p<0.01$). + expressed per mg of microsomal protein.*

Table 2. Effect of phenobarbital (PB), dexamethasone (DEX) and β-naphtoflavone (BNF) on rat liver microsomal ethoxyresorufin deethylase (EROD), pentoxyresorufin depentylase (PROD) and ethoxycoumarin deethylase (ECDE) activities. Microsomes were prepared from the livers of 3 animals in each group

Group	EROD pmol/(mg.min)$^{-1}$	PROD pmol/(mg.min)$^{-1}$	ECDE nmol/(mg.min)$^{-1}$
Control			
Corn oil	84.1±23.4	24.8±7.2	344±129
0.9 % NaCl	67.5±14.9	32.3±10.5	458±103
Induced			
PB	618±444	937±68**	3473±829*
DEX	2983±408**	84.0±38.6	2630±704*
BNF	14274±1158**	34.1±17.6	9461±2100*

*Data are means±S.D. Significant difference from control (Mann-Whitney test, * $p<0.05$, ** $p<0.01$). The enzyme activities are expressed per mg of microsomal protein.*

Table 3. Cytochrome P450 content, microsomal ethoxyresorufin deethylase (EROD) and ethoxycoumarin deethylase (ECDE) activities in two human livers.

	Cytochrome P450 (nmol/mg)	EROD+ pmol/(mg.min)$^{-1}$	ECDE+ nmol/(mg.min)$^{-1}$
HL01	0.30	125	168
HL02	0.21	28	137

+ expressed per mg of microsomal protein.

Table 4. Effect of methotrexate (MTX) on the activity of ethoxyresorufin deethylase (EROD), pentoxyresorufin depentylase (PROD) and ethoxycoumarin deethylase (ECDE) in rat liver microsomes prepared from control (C), phenobarbital (PB), dexamethasone (DEX) and β-naphtoflavone (BNF) pretreated animals.

Enzyme	Group	MTX (μM) 0	0.5	5	50	500
EROD	C	100	99±0.8	96±0.8	101±5.0	96±1.3
	PB	100	97±4.8	98±8.2	95±5.4	97±6.1
	DEX	100	109±3.9	102±1.6	101±2.6	97±1.4
	BNF	100	94±6.6	94±0.8	104±6.4	103±9.5
PROD	C	100	97±1.3	98±2.1	100±4.6	98±0.9
	PB	100	97±1.4	95±2.1	95±1.6	98±2.1
	DEX	100	103±2.2	100±0.8	98±0.5	99±1.3
	BNF	100	97±2.2	100±4.3	96±1.7	93±1.7
ECDE	C	100	101±0.8	102±2.1	93±1.5	84±2.6
	PB	100	101±2.2	107±2.9	99±1.2	94±0.9
	DEX	100	97±1.7	96±2.0	97±1.0	88±4.4
	BNF	100	103±1.5	102±2.0	101±1.6	99±0.8

Data are means±S.D. of 3 incubations. The activity is expressed in percentage of the control activity (without MTX in the incubation mixture).

Table 5. Effect of methotrexate (MTX) on the activity of ethoxyresorufin deethylase (EROD) and ethoxycoumarin deethylase (ECDE) in human liver microsomes.

Enzyme	MTX (μM) 0	0.5	5	50	500
EROD	100	99±7.6	97±4.2	98±6.8	93±5.7
ECDE	100	98±6.2	98±4.1	99±11.7	80±11.4

Data are means±S.D. of 3 incubations. The activity is expressed in percentage of the control activity (without MTX in the incubation mixture).

We did not find any formation of 7-OHMTX in microsomes from the rat liver (either induced or control) or from the human liver. In both species, enzymatic activities associated with cytochrome P450 (EROD, PROD, ECDE) were not inhibited by 0.5–500 μM MTX (Tables 4 and 5). The measurement of ECDE activity in the presence of 500 μM MTX was affected by the strong spectral interference of MTX at the excitation wavelength, causing less reproducible results. Therefore, a small degree of ECDE inhibition observed in microsomes from control rats and the human liver at this MTX concentration could be an artifact. In separate experiments, we used quinidine and metyrapone in order to demonstrate the inhibition of monooxygenase activities in the rat liver induced by PB and to validate our methods (Fig. 1).

In the cytosol from the rat and human liver, MTX was converted to 7-OHMTX at a rate which was constant for 30 min after the addition of MTX to the medium and comparable for both species (Fig. 2). The rate of MTX hydroxylation in the cytosolic fraction of the human liver was reduced by 31.5 % in the presence of allopurinol at the equimolar concentration. This inhibition effect of allopurinol was not observed in the cytosol of the rat liver. Menadion decreased the rate of MTX hydroxylation in the cytosol of human and rat livers by 69 % and by 94 %, respectively (Fig. 2).

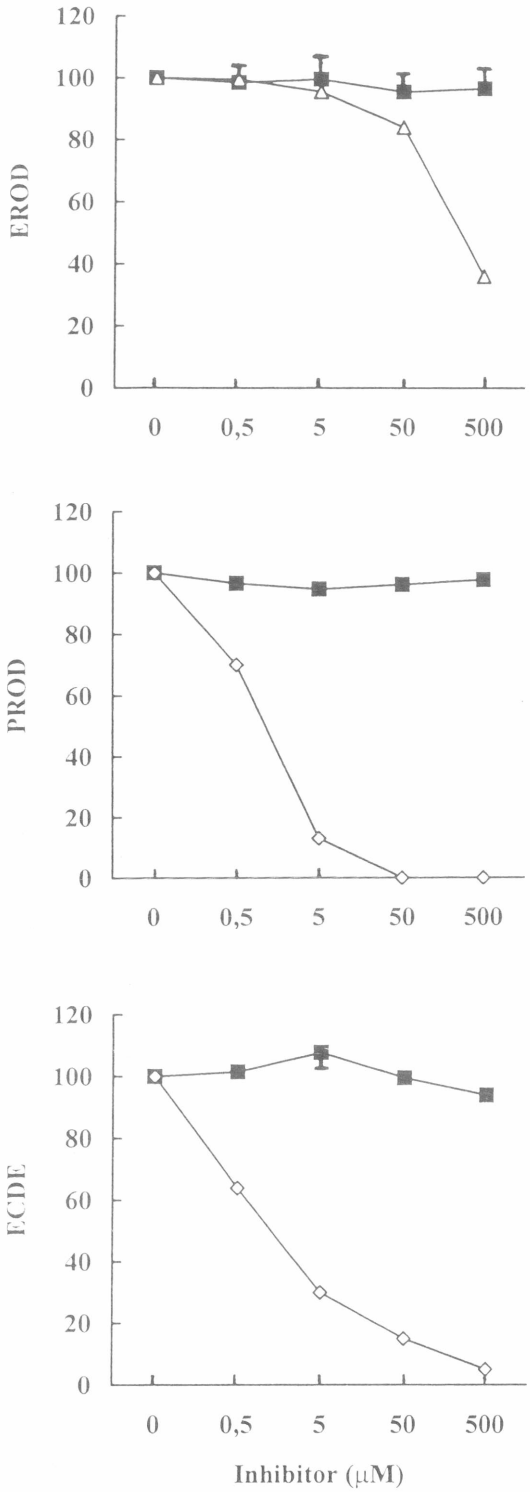

Fig. 1. *Inhibition of ethoxyresorufin deethylase (upper panel), pentoxyresorufin depentylase (middle panel) and ethoxycoumarin deethylase (lower panel) activities by methotrexate (full square, N=3) and by model inhibitors quinidine (open triangle, N=1) and metyrapone (open rhombus, N=1) in liver microsomes from phenobarbital-pretreated rats. The activity is expressed in percentage of control activity (without an inhibitor in reaction mixture). Data are means ± S.D.*

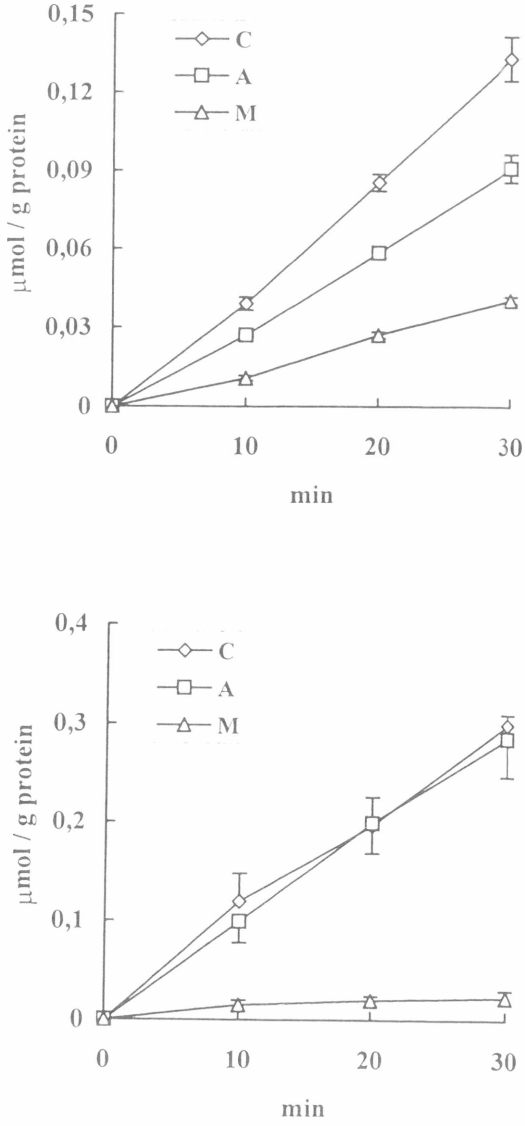

Fig. 2. *7-hydroxymethotrexate formation in cytosolic fraction of human (upper panel) and rat (lower panel) liver incubated with 100 μM methotrexate at 37 °C. The cytosolic fraction was preincubated with vehicle (C), 100 μM alllopurinol (A) and 100 μM menadion (M) before the addition of methotrexate. Data represent the mean ± S.D. of 3 incubations.*

Discussion

Cytochrome P450 represents a very complex superfamily of enzymes which are responsible for the biotransformation of endogenous compounds (steroids) as well as of exogenous ones (drugs, procarcinogens, promutagens) (Relling *et al.* 1992). Relatively simple inhibition studies can be used either for obtaining preliminary information about cytochrome P450-associated metabolic pathways or for predicting drug interactions. The combined use of various inducers and inhibitors, followed by the conversion of several substrates, serves as a finger-print

for a specific isoenzyme in animal models (Pasanen *et al.* 1988). The O-dealkylations of ER, PR and EC are widely used activity probes for measuring the cytochrome P450-isozymes and their induction by xenobiotics. In comparison with the non-induced condition, the induction of CYP450 by PB, BNF and DEX causes a shift in the relative contribution of different isoforms in the conversion of metabolic probes. Thus, ER is metabolized by CYP 2C6 and 2B1 in non-induced microsomes from the rat liver but is highly selective for CYP 1A1 after pretreatment of the rats with BNF. Similarly, PR is metabolized by several isoforms in non-induced livers, but mainly by CYP 2B1 in PB-induced rat livers and by CYP 1A in BNF-induced ones (Burke *et al.* 1994). We have therefore used classical inducers of cytochrome P450 to investigate the effect of MTX on the rate of conversion of the metabolic probes by isoforms which differ from those in non-induced rats. There exist species differences and similarities in organ distribution, catalytic activity, substrate specificity, inducibility and regulation of CYP450 s. In healthy human liver, CYP 3A4 is the most abundant isoform with about 25 % of the total amount of cytochrome P450, whereas CYP 1A2, CYP 2C8 and CYP 2C9 represent around 10 % each (Breimer 1995). In the liver of male rats, the main isoform is CYP 2C11 (50 %) followed by CYP 3A2 and CYP 2C6 (Guitton *et al.* 1994). ER is metabolized by CYP1A in man as well as the rat. N-deethylation of lidocaine is catalyzed in man and rat by CYP3A and CYP2C, respectively. The conversion of EC was catalyzed by 10 of 11 isoforms of human cytochrome P450 studied by Waxman *et al.* (1991).

As described by Pelkonen and Breimer (1994), we observed an increase in the content of cytochrome P450 in the group of induced rats as compared to those pretreated with either 0.9 % NaCl or corn oil. PB-induction in rats was characterized by a large and selective elevation of PROD activity (24-fold) as compared to only a modest increase of EROD (6-fold) and ECDE (8-fold). The pretreatment of rats with BNF resulted in the highest induction of EROD observed in our experiments (170-fold) and a marked increase of ECDE (21-fold) activity. DEX-induction caused a large elevation of EROD activity (36-fold), whereas activities of PROD and ECDE were increased only slightly (3-5 fold). A similar induction pattern was observed by others (Burke *et al.* 1985, Lubet *et al.* 1985, Mayer *et al.* 1990, Nerurkar *et al.* 1993).

The cytochrome P450 concentration in microsomes prepared from the two samples of human liver agreed well with values published by Forrester *et al.* (1992). ECDE activity was comparable to the results of Pasanen *et al.* (1986) for homogenates of human liver. EROD activity agreed well with values published by Wrighton *et al.* (1993) and Birgersson *et al.* (1985).

A lack of any MTX hydroxylating activity in the microsomal fraction of the rat and human liver and the formation of the metabolite in the cytosolic liver fraction of both species provides clear evidence that MTX is oxidized by a soluble enzymatic system. Because cytochrome P450 resides mainly in the microsomal fraction, it seems that MTX hydroxylation is not catalyzed by cytochrome P450 either in man or in the rat. Furthermore, MTX did not inhibit EROD, PROD and ECDE activities in rat liver microsomes (non-induced or induced) and EROD and ECDE activities in human liver microsomes. Inhibition of cytochrome P450 in the rat liver frequently observed *in vivo* (Guitton *et al.* 1994, Lukienko *et al.* 1993) as well as the reduced clearance of theophylline in man (Glyn-Barnhart *et al.* 1991) after MTX administration can most probably be explained by an indirect inhibitory action of MTX (decreased CYP450 synthesis). The extent of the inhibition is most likely enhanced by inflammation, fasting and stress associated with the administration of a cytostatic agent (Guitton *et al.* 1994).

In rabbits, ALO was identified to be a cytosolic MTX-hydroxylase. The MTX hydroxylating ability of the rat and human liver is comparable and the rat is a suitable animal model of hepatic MTX status in man. However, partially purified preparations of ALO from the human and rat liver have been found to exhibit negligible activity towards MTX (Newton *et al.* 1984). The specific activity of ALO is very low in extracts of human tissues (Krenitsky *et al.* 1986). Rats are known to have higher activities of ALO in liver than humans (Jones *et al.* 1987). ALO and XO are structurally similar cytosolic enzymes with broad substrate specificity which oxidize nitrogen-containing heterocycles, and metabolize many drugs. The levels of XO, from species to species, are within the same order of magnitude. In contrast, the levels of ALO vary by three orders of magnitude. Bremnes *et al.* (1991) described a strong inhibition of 7-OHMTX formation in rat *in vivo* and *in vitro* by amsacrine, the most potent inhibitor of ALO currently known. Yu *et al.* (1989) found no effect of the XO inhibitor allopurinol and the aldehyde dehydrogenase inhibitor cyanamide on 7-OHMTX production in the rat. However, XO may have more important role in the metabolism of MTX in human tissues. To test this hypothesis, we preincubated menadion (an inhibitor of ALO) and allopurinol (an inhibitor of XO) in the hepatic cytosol before adding MTX in equimolar concentrations. Our findings that a part of MTX hydroxylating activity in the cytosolic fraction of the human liver is susceptible to inhibition by allopurinol and that it remained active in the presence menadion, support this hypothesis. Therefore, our next interest in this field will be focused on MTX hydroxylation by purified XO preparations from the human liver and intestine.

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Abbreviations used:
ALO – aldehyde oxidase, BNF – β-naphtoflavone, DEX – dexamethasone, MTX – methotrexate, ECDE – ethoxycoumarin deethylase, EROD – ethoxyresorufin deethylase, 7-OHMTX – 7-hydroxymethotrexate, PB – phenobarbital, PROD – pentoxyresorufin depentylase, XO – xanthine oxidase

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