Lipoperoxidation Rates and Drug-Oxidizing Enzyme Activities in the Liver and Placenta of some Mammal Species During the Perinatal Period

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Summary. Lipoperoxidation and drug-metabolizing enzymes were measured in livers and placentas of different mammal species during the perinatal period. In placentas and fetal livers of rat, rabbit and guinea-pig, cofactor-supported lipoperoxidation was negligible, as were the activities of drug-oxidizing enzymes. Human fetal liver contained an intact drug-oxidizing electron transport chain, and lipoperoxidation activity was accordingly observed. It is suggested that lesions mediated by lipoperoxidation may be possible in human fetus, but they are less probable in animal fetuses.

Several reports have been published on the development of the drug metabolizing capacity in different animal species during the fetal and newborn periods[3]. These studies show that the negligible drug metabolizing activity in animal fetuses increases after birth at different rates, depending on the species, the type of reaction and the substrate used. Although the animal fetus shows almost no drug metabolizing activity, the human fetus was found to metabolize drugs and other foreign compounds in quantities of almost the same magnitude as do adult laboratory animals[4–6].

Peroxidation of liver lipids, which has been shown to be closely connected with the NADPH-depending microsomal electron transport chain[7,8], is responsible for many different lesions and processes, e.g. drug-induced liver injura[9]. The peroxidation activity is also responsible for interacting with the lipids of the endoplasmic reticulum, thus decreasing the drug metabolizing capacity of the liver[10]. It is of importance to know the perinatal development of lipid peroxidation in order to evaluate the possible role of this reaction in perinatal tissue injuries.

With the above-mentioned goal in mind, we compared the lipoperoxidation rates and the microsomal drug metabolizing capacity in the fetal, newborn and maternal rat, rabbit and guinea-pig liver microsomes and in 3 human fetuses.

Materials and methods. The animals studied were outbred rats of Sprague-Dawley strain, white New-Zealand rabbits and guinea-pigs obtained from Orion, Mankkaa, Finland. The human fetuses were obtained from the Department of Obstetrics and Gynecology, University of Oulu.

Preparation of tissues. All animal and fetal tissue samples were processed as described in a previous paper[6]: 20% liver and placenta homogenates were prepared in

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Table I. Drug-oxidizing enzymes in the fetal, newborn, and maternal liver microsomes and in the placentas of rat, rabbit and guinea-pig compared to human fetal liver microsomes

Species	*n*	P-450 (nmol/mg)	cyt. C-reduction (nmol/mg × min)	Aminopyrine demethylase (nmol/g × min)
Rat				
Fetal (end-term)	(3)[a]	0.062	17	0.5
Newborn (2–4 days)	(3)[a]	0.18	25	0.8
Maternal liver	(7)[b]	0.44±0.12	95±21	48,5±9.7
Placenta (end-term)	(3)[a]	0.03	11	0.5
Rabbit				
Fetal (end-term)	(2)[a]	0.082	24	4.5
Newborn (1–3 days)	(6)[b]	0.17±0.04	40±5	10.9±2.2
Maternal liver	(3)[b]	0.82	124	90.6
Placenta (end-term)	(2)[a]	0.03	5	0.5
Guinea-pig				
Fetal	(7)[b]	0.12±0.09	11±2	–
Newborn (1–3 days)	(3)[b]	0.44	80	–
Maternal liver	(2)[b]	0.85	87	–
Placenta (end-term)	(7)[b]	0.03±0.08	5	–
Human				
Fetal	(3)[b]	0.18	35	21.8

[a] Number of litters studied; livers or placentas from each litter were pooled, homogenized and enzymes determined. [b] Indicates individuals.

Table II. Lipoperoxidation activity in various mammal species at the perinatal period

	n	No addition	+ Cofactors	+ Ascorbic acid
Rat				
Fetal	(3)[a]	0.016±0.004	0.044±0.011	0.259±0.056
Newborn	(3)[a]	0.018±0.008	0.288±0.045	0.523±0.078
Maternal	(7)[b]	0.022±0.006	0.339±0.029	0.950±0.077
Placenta	(3)[a]	0.044±0.018	0.027±0.004	0.067±0.09
Rabbit				
Fetal	(2)[a]	0.013±0.003	0.048±0.003	0.230±0.030
Newborn	(6)[b]	0.010±0.003	0.122±0.045	0.084±0.041
Maternal	(3)[b]	0.018±0.008	0.220±0.110	0.443±0.118
Placenta	(2)[a]	0.018±0.003	0.020±0.005	0.030±0.010
Guinea-pig				
Fetal	(7)[b]	0.004±0.002	0.026±0.005	0.076±0.010
Newborn	(3)[b]	0.006±0.002	0.088±0.032	0.063±0.015
Maternal	(2)[b]	0.014±0.005	0.134±0.021	0.270±0.080
Placenta	(7)[b]	0.002±0.001	0.002±0.001	0.043±0.014
Human				
Fetal	(3)[b]	0.012±0.003	0.131±0.028	0.382±0.172

The peroxidation activity is expressed as O. D. $_{530\,nm}$ of the TBA reactants ± SEM.
[a] Number of litters studied. [b] Indicates individuals.

0.1 *M* phosphate buffer, pH 7.4, using a Teflon-glass Potter-Elvehjem homogenizer. In the preparation of animal fetal tissues, livers and placentas were pooled to obtain sufficient amounts for analysis. Homogenates were centrifuged at 12,000 × *g* for 20 min, and the supernatants thus obtained were submitted to 100,000 × *g* for 60 min in order to obtain the microsomal fraction and the soluble fraction.

Assay methods. The amount of cytochrome P-450 and the activities of NADPH-cytochrome-C-reductase and aminopyrine demethylase were determined as reported before[6, 11, 12]. The lipoperoxidation rates were determined by the thiobarbituric acid system[13] with in vitro additions of cofactors or ascorbic acid as indicated in the Tables. The incubation volume consisted of the same amount of cofactors as in the enzyme assays, (134 μmol KCl, 6.7 μmol $MgCl_2$, 59 Wrobl. units of glucose-6-phosphate dehydrogenase, Fluka, or 2 μ*M* ascorbic acid). The reaction was started with 0.1 ml of microsomal suspension which corresponds to 100 mg of tissue. The final volume was made up to 2 ml with the buffer. The reaction was stopped after 30 min with 0.5 ml of 25% TCA and the thiobarbituric acid reactants formed determined in 1 ml of the supernatant.

Protein concentrations were made with the Biuret method[14], using bovine serum albumine as a reference standard.

Results and discussion. The activities of drug-oxidizing enzymes in the human fetal liver and in the fetal, newborn and maternal liver microsomes and placentas of rat, rabbit and guinea-pig are shown in Table I. The cytochrome P-450 was detectable in all fetal tissues studied and its concentration gradually increased after birth. The concentrations of cytochrome P-450 correlate with the rates of oxidative metabolism after birth. This indicates that the microsomal content of cytochrome P-450 may be taken as a rate-limiting factor to the development of oxidative metabolism of drugs by the liver. In agreement with the earlier studies[6], the concentration of cytochrome P-450 was highest in human fetal liver.

In the present study the aminopyrine demethylase activity follows the increase in age and the amount of cytochrome P-450. No sudden increase in enzyme activity can be seen after birth as reported by KUENTZIG et al.[15] who studied the aryl hydrocarbon hydroxylase activity in newborn guinea-pigs. DUTTON[16] and HENDERSON[17] report also a sudden elevation of the UDP-glucuronyl-transferase activity in rat liver homogenates just after birth. BAKKEN and FOG[18] supposed that the accumulation of unconjugated bilirubin might act as a trigger for this conjugating system. The possible trigger for other drug metabolizing enzymes has not been found.

The activity of the flavoenzyme in the electron-transport chain, NADPH-cytochrome-C-reductase, also increases parallel with the concentration of cytochrome P-450. Although the newborn guinea-pigs show a relatively poor metabolic activity, they have a high concentration of cytochrome P-450 and NADPH-cytochrome-C-reductase activity as compared to other newborn animals.

All the animal placentas studied showed only traces of drug metabolizing enzyme activities, which is in agreement with the finding that significant activities can be observed only in placentas of smoking mothers or in those of pregnant animals treated with polycyclic aromatic hydrocarbons[19–22].

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The lipoperoxidation rates are given in Table II. The values with added cofactors correspond well with the enzyme activities shown in Table I, thus reflecting the close connection with the NADPH-depending microsomal electron transport chain. The rate of cofactor-supported lipoperoxidation in human fetal liver was 3 to 5 times greater when compared to fetuses of other mammalian species studied. This is in agreement with the higher content of cytochrome P-450 and higher activities of related enzymes in human fetal liver. Furthermore, it must be taken into consideration that animal fetuses were end-term ones, whereas human fetuses were about 14- to 16-weeks of fetal age, i.e., from the first half of pregnancy.

Although the activation of the lipoperoxidation by ascorbic acid is known to be non-enzymatic[23], the values found rely on the enzyme activities. The reason why the fetal and newborn guinea-pig show less lipoperoxidation activity than to those of other species even after addition of ascorbic acid remains obscure. It may be due to the fact that guinea-pig differs from rat and rabbit in the ascorbic acid metabolism by lacking the microsomal enzyme L-gulonolactone oxidase.

Our results show that fetuses of common laboratory animals have only negligible levels of drug-metabolizing enzymes and cofactor-supported lipoperoxidation. On the other hand, the human fetal liver contains a typical intact electron transport chain and actively supporting lipid peroxidation. The suggested role of lipoperoxidation in drug-induced tissue lesions, e.g. in CCl_4-induced liver injury, permits the speculation that animal fetuses are resistant to those kinds of injuries, whereas the human fetus may develop lipoperoxidation-mediated tissue lesions because of the presence of an intact electron transport chain in liver microsomes.

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The Effect of Thiazol-4-ylmethoxyamine, a Histidine Decarboxylase Inhibitor, on the Development of Morphine Tolerance and Physical Dependance in Mice[1]

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Summary. A new histidine decarboxylase inhibitor, thiazol-4-ylmethoxyamine (TMA), injected into mice in a dose of 100 mg/kg i.p. 48 h before the implantation of a morphine-containing pellet, inhibited the development of morphine tolerance and physical dependence.

General inhibitors of protein synthesis, e.g. cycloheximide, are known to impair the development of morphine tolerance and physical dependence[3,4]. This action is presumably related, at least in part, to a depression of synthesis of enzymes involved in the metabolism of neurotransmitters which mediate these central actions of morphine. There is increasing evidence that histamine (Hm) may be a transmitter in the brain[5], and some recent work appears to implicate Hm in the neural mechanisms of morphine tolerance and physical dependence[6–8]. If this is so, then the specific and potent histidine decarboxylase inhibitor thiazol-4-ylmethoxyamine (TMA)[9,10], which can cross the blood-brain barrier, should have actions rather similar to cycloheximide in this respect.

Materials and methods. Mice (WHT/Ht strain; both sexes, weighing 25–40 g, in roughly equal numbers) were

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Table I. Effect of TMA on the analgesic action of morphine in naive and oependent mice[a]

Treatment	Naive ($n=25$)		Dependent ($n=24$)		Slope Ratio	Potency Ratio	$f_{P.R.}$
	Slope function	AD_{50} (mg/kg)	Slope function	AD_{50} (mg/kg)		(P. R.)	
Control group	2.02 (2.46–1.66)	6.85 (8.84–5.31)	2.27 (3.97–1.30)	17.5 (39.8–7.7)	1.12 (2.02–0.68)	2.55 (4.72–1.38)	1.85
TMA group	1.23 (1.62–1.08)	9.20 (11.73–7.22)	2.02 (3.22–1.30)	11.00 (18.15–6.67)	1.64 (2.83–0.95)	1.20[b] (2.1–0.69)	1.75

[a] The potency is expressed as the median analgesic dose (AD_{50}) of morphine after morphine-containing and blank pellet implantation. Figures in parantheses denote 95% confidence limits.
[b] As the P. R. is smaller than the $f_{P.R.}$, the 2 AD_{50} values are not significantly different.
Calculations according to the methods of LITCHFIELD and WILCOXON[17]