IN VIVO AND IN VITRO INDUCTION OF P450 ENZYMES IN REGENERATING LIVER CELLS

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**Introduction**

Cytochrome P450 (P450) enzymes catalyze biotransformation of various exogenous (drugs, chemicals, pesticides) and endogenous (prostaglandines, steroid hormones, leukotriens) substrates. Most of the P450s, which are involved in drug metabolism, are expressed in the liver (1). Regulation of P450 enzymes is mediated by endogenous regulatory factors (endogenous regulatory mechanism) or by exogenous molecules (inducers). Mostly, nuclear receptors (aromatic hydrocarbon receptor (AhR), constitutive androstane receptor (CAR), pregnane X receptor (PXR)) are involved in regulation. In the nucleus, the ligand activated nuclear receptor binds to the heterodimerization partner (retinoid X receptor, RXR) and the complex activates the responsive element of the gene.

In addition several co-activators and co-repressors can modulate the effect (2, 3, 4).

Beside inducers and endogenous regulatory molecules there are some other changes, like cell proliferation, tissue damage, chirrhosis, which can influence P450
activities in the liver. After necrosis the liver cells start to proliferate. An important model of hepatocyte proliferation is liver regeneration after partial hepatectomy.

Decrease in monooxygenase activities during liver regeneration in rats was first described by Von der Decken and Hutlin in 1960 (5). Number of investigators have reported that P450 activities, apoproteins and mRNA levels are reduced during regeneration (6, 7). There are many explanations for the apparent decrease of drug metabolism associated with regeneration: 1. surgical stress, 2. increase in heme oxygenase activity (8) 3. replication, but not transcription is the most important mission of DNA (9) 4. dedifferentiation of liver cells, 5. increasing level of NO (nitrogen monoxide) reduces P450 activity by binding to active center of the enzyme (10).

After partial hepatectomy, the inducibility of some P450s also changes, because regeneration alters the levels of regulatory molecules involved in P450 induction (e.g. RXR) (11). Another example is aromatic hydrocarbone
receptor. Its level also changes during cell proliferation (12).

**Object of our study**

Our work investigated the effect of partial hepatectomy on the basal level and inducibility of CYP1A, CYP2B, CYP2E1 and CYP3A, which are mostly involved in drug metabolism.

The aim of our first study was to detect P450s on the basis of selective enzyme activities and protein amount, and to investigate the effect of dexamethasone treatment during liver regeneration. The following questions were answered:

- Whether CYP1A, CYP2B, CYP2E1 and CYP3A enzymes changed during early period of liver regeneration (0-72h)?
- Whether in vivo treatment of dexamethasone - a synthetic glucocorticoid - modulates the P450 levels during liver regeneration?
2. The inducibility of CYP1A, CYP2E1 and CYP3A was also measured in hepatocytes isolated from regenerating liver. Our investigations answer:
Are 3-methylcholanthrene, imidazole, and dexamethasone treatment able to change the activities and protein levels of CYP1A, CYP2E1 and CYP3A in hepatocytes isolated from regenerating liver?
Is there any difference in the inducibility of these enzymes analyzed in cells from regenerating liver compared to the activities and protein levels measured in the hepatocytes from sham operated rats?

For measuring CYP2E1 in cell culture we have adapted a selective method for detecting CYP2E1 activity (chlorzoxazone 6-hydroxylase measurement).
Methods

Operation:

Partial hepatectomy: about the two-thirds of the liver was removed by a method of Higgins and Anderson (13).

Liver perfusion: calcium exemption, digestion with collagenase (14).

Preparation of microsomes:

Microsomes were prepared by differential centrifugation from the rat livers and also from cultured hepatocytes.

Enzyme activities:

Enzyme activities were measured from microsomes and cell culture systems as follows by specific substrates for each enzyme:

CYP1A; Ethoxyresorufine O-dealkylase activity (15)
CYP2B; Pentoxyresorufine O-dealkylase activity (15)
CYP2E1; p-Nitrophenol-hydroxylase activity (16)
        Ethoxycoumarine O-dealkylase activity (17)
        Chlorozaxzone 6-hydroxylase activity (18)
CYP3A; Aminopirine N-demethylase activity (19)
Ethylmorphine N-demethylase activity (20)

**Western blot analysis:**
Protein amounts of enzymes were determined from microsomes prepared from cells and livers. Microosomal proteins were separated in SDS-polyacrilamide gel (7.5%) using method of Laemmli (21). After blotting to nitrocellulose membrane (22) the isoenzymes were detected with specific primary and secondary antibodies using horseradish peroxydase reaction (23).

**Statitics:**
Statistical analyses of the data were carried out by using Student’s t-test. Level of significance was p<0.05.
Results and discussion

In the first experiment the changes of P450s were studied after partial hepatectomy. The results were as follows:
1. During 72h regeneration after partial hepatectomy:
   the level of CYP1A decreased in the early hours and then it was the same like in the sham operated animals.
   the activity and protein amount of CYP2B also decreased, and no recovery was observed during 72h period.
   the activity of CYP2E1 showed fluctuation in the early hours of regeneration, although the protein levels did not change. In the late hours the activity returned to the levels measured in the sham operated animals.
   In the case of CYP3A there was no change in the early hours of regeneration, but after 24 hours a drastic decrease in the activity and protein level was observed.
2. Dexamethasone administration, which alters both regeneration process and activities of CYP1A and CYP3A, could modulate the activities of some P450s after partial hepatectomy:

Dexamethasone treatment diminished the decrease of the enzyme activity and protein levels of CYP1A and CYP2B after partial hepatectomy. Dexamethasone treatment stabilized the CYP2E1 enzyme in the first 6 h, no fluctuation was observed in the enzyme activity. Dexamethasone prevented the loss of CYP3A enzyme activity and protein during early hours of regeneration.

3. In the third experiment, the inducibility of P450 enzymes (CYP1A, CYP2E1 and CYP3A) was investigated in cells isolated from regenerating liver. These cells retained P450 inducibility, but the level of induction changed: The inducibility of CYP1A in 3-methylcholantrene treated cells was higher (3x) than in hepatocytes from sham operated animals. Both the activity and protein level increased. The degree of CYP2E1 induction by imidazole in the cells isolated from regenerating liver was the same as in the hepatocytes obtained from sham operated animals (2-4x).
The inducibility of CYP3A by dexamethasone in cells isolated from 1, 3 and 7 day long regenerating liver also differed from that in hepatocytes of sham operated rats; it was 2-2.5 fold higher in dexamethasone treated cells than in normal, induced cells.

Summarizing our results, partial hepatectomy altered the basal activity and protein levels of P450 enzymes. The activities of CYP1A, CYP2B, CYP2E1 and CYP3A decreased in the early hours of regeneration, but the rate of the reduction depends on the type of the enzyme. Dexamethasone could modulate these changes. The rate of induction of P450s in cells isolated from regenerating liver also changed compared to the cells isolated from sham operated animals. The enzymes were inducible in cells isolated from regenerating liver, but the rate of induction of CYP1A and CYP3A was higher in cells from regenerating liver, than in hepatocytes isolated from sham-operated animals.
References:


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Publications


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