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PRELIMINARY COMMUNICATION

ENHANCED BIOTRANSFORMATION OF NITROFURANTOIN IN RATS AFTER INDUCTION WITH 3-METHYLCHOLANTHRENE OR ß-NAPHTHOFLAVONE

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Hepatic biotransformation of nitrofurantoin (NF) is mainly due to reductive processes (1, 2). Oxygen leads to an almost complete inhibition of the reductive metabolism of NF (3) and does not promote any known oxidative metabolism of this antibiotic as catalyzed by liver tissues. Accordingly, identified metabolites of NF mainly confer to reduced products (2). An oxidized metabolite could only tentatively be identified in the urine of rats fed NF (4). In accordance with these results we previously found, that the elimination of NF in the isolated rat liver is rather low under aerobic conditions but is enhanced severalfold under an anaerobic atmosphere (5).

In this study we want to report that pretreatment of rats with 3-methylcholanthrene (MC) or ß-naphthoflavone (ßNF) – but not with phenobarbital (PB) – leads to an enhanced hepatic clearance of NF in the isolated liver under aerobic conditions and to a decrease of the urinary excretion of unchangend drug after an oral dose of NF. The induction by MC of ßNF also causes the formation of a yellow, polar metabolite of NF in perfusate as well as in urine which might prove as a suitable *in vivo*-indicator of the cytochrome "P-448"-dependent induction state. Though the chemical structure of this metabolite is still unknown there are some similarities with the 4-hydroxy-5-nitrofuran derivative as described by Olivard et al. (4).

The results reported herein were briefly presented at a national meeting (6).

MATERIAL AND METHODS

Male, Sprague-Dawley rats weighing 250-300 g were used in all experiments. Pretreatment with PB was accomplished by i.p. application of 3 x 80 mg PB/kg at 24 h intervals. For pretreatment with MC 3 x 20 mg/kg were given i.p. at 12 h intervals. ßNF was given i.p. as a single dose of 80 mg/kg in case of perfusion experiments; 2 x 100 mg ßNF/kg were given in case of *in vivo*-experiments. The last injection was given 24 h before the perfusion experiment in case of PB and ßNF and 36 h in case of MC. For *in vivo*-experiments the time interval between the last injection and the oral dosing of NF was 24, 36 and 12 h for PB, MC and ßNF, respectively. The group of control animals consisted of untreated, saline- and oil-treated rats since no differences concerning the metabolism of NF could be detected. Rat liver perfusion was performed as described earlier (7) in a recirculating mode at a constant flow of 30 ml/min at 25 ^{o}C for a period of 40 min using a perfusion medium of 100 ml Krebs-Ringer bicarbonate buffer containing 400 mg glucose and 2 g of bovine serum albumin (Behringwerke, Marburg) but omitting erythrocytes. NF (Sigma Chemical Co., St. Louis, MO) was used at an initial concentration of 0.1 mM. The content of NF in perfusate and urine was determined by the method of Conklin and Hollifield (8). The spectrophotometric determination is based on extraction of NF into nitromethane followed by the formation of a yellow complex with hyamine hydrochloride. An interference with metabolites of NF could be excluded. The hepatic clearance of NF was calculated according to Rowland (9). Formation of a yellow, polar metabolite of NF

was determined by the change of absorbance at 415 nm in the aqueous phase after extraction of perfusate or urine with nitromethane at acidic pH.

Under *in vivo*-conditions NF was given as a single oral dose of 33 mg/kg in a 0.5 % suspension of gum arabic. Animals were housed in metabolism cages and urines were collected for a 24 h period.

Microsomes were prepared as described earlier (10). Benzo(a)pyrene hydroxylation was measured according to Gielen et al. (11). Absorbance spectra of the perfusate were recorded on a Perkin-Elmer 356 spectrophotometer.

RESULTS

Under aerobic conditions NF metabolism is rather low in the isolated liver derived from control animals leading to a hepatic clearance of 0.9 ml/min (Table 1). Pretreatment with PB does not alter the hepatic elimination of NF in a significant manner. Accordingly, the urinary excretion of unchanged NF reaching 26 % of dose in 24 h urine under control conditions is not altered by PB (Table 1) as shown before by Veronese et al. (12). Pretreatment with MC or βNF, however, leads to a significant enhancement of hepatic elimination of NF. The excretion of unchanged NF in 24 h urine is significantly decreased by these inducers suggesting an enhanced metabolism also under *in vivo*-conditions.

When the hepatic clearance of NF was taken as a function of microsomal benzo(a)pyrene hydroxylase activity as determined in microsomes derived from the corresponding livers there is a strong correlation (Figure 1).

Table 1: Influence of induction on the biotransformation of nitrofurantoin in the isolated perfused rat liver and *in vivo* after an oral dose of nitrofurantoin to rats

Pretreatment	Liver Perfusion		In vivo	
	Elimination[a]	Formation of polar metabolite[b]	Elimination[c]	Formation of polar metabolite[d]
Control	0.9 ± 0.2 (8)	0.5 ± 0.1 (5)	25.9 ± 2.0 (11)	1.3 ± 0.2 (5)
Phenobarbital	1.4 ± 0.3 (4)	0.8 (1)	25.5 ± 2.2 (3)	0.7 ± 0.1 (3)
3-Methyl-cholanthrene	4.7 ± 0.2** (8)	26.9 ± 1.3** (5)	5.0 ± 0.7** (7)	7.6 ± 1.1** (4)
β-Naphthoflavone	3.1 ± 0.3* (3)	19.9 ± 4.3* (3)	5.5 ± 2.3* (3)	7.1 ± 1.4* (3)

Initial nitrofurantoin concentration was 0.1 mM in rat liver perfusion experiments. For *in vivo*-experiments nitrofurantoin was given as an oral dose of 33 mg/kg. Induction was achieved as given under Material and Methods. Unmetabolized nitrofurantoin was determined according to Conklin and Hollifield (8). Elimination in liver perfusion experiments is given as (a) the hepatic clearance of nitrofurantoin (ml x min^{-1}). Under *in vivo*-conditions the elimination is expressed as (c) the % of administered dose excreted as unchanged nitrofurantoin in 24 h urine. The formation of polar metabolite was calculated from the absorbance change at 415 nm in the aqueous phase after extraction with nitromethane and is given as (b) OD. 10^{-3} x ml $perfusate^{-1}$ x min^{-1} for perfusion experiments and as (d) OD. 10^{3} x 24 h $urine^{-1}$ x mg administered $dose^{-1}$. Values are means ± S.E.M. except one single determination. P (vs. Control; Student's *t*-test) * < 0.005; ** < 0.001.

NF exerts an absorbance maximum at 388 nm in the visible region after addition to the perfusion medium (Figure 2). After 40 min aerobic perfusion passing the liver of an untreated animal the wavelength of the absorbance maximum is not altered; the extent is slightly reduced according to the minimal elimination of NF under these conditions. Anoxic liver perfusion leads to the expected disappearance of the absorbance characteristics of NF as caused by the marked reductive metabolism of NF under anaerobic conditions. Aerobic liver perfusion

with NF after previous induction with MC, however, leads to the formation of a new absorbance maximum at 415 nm.

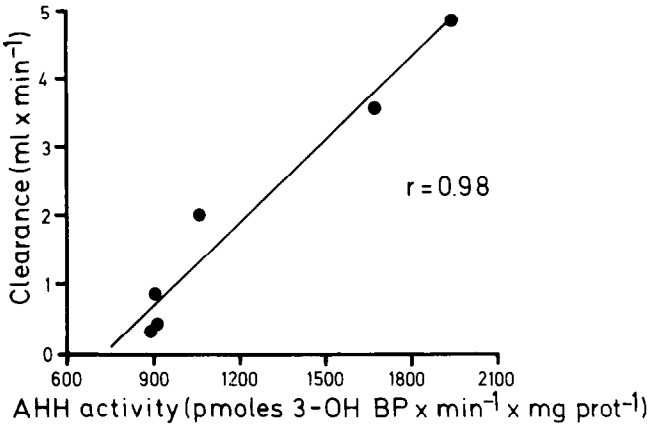

Figure 1: Hepatic clearance of nitrofurantoin as a function of AHH activity.
Liver perfusions were performed at various induction states and hepatic clearance of nitrofurantoin was calculated as given by the ordinate. Afterwards microsomes were prepared from those livers and microsomal benzo(a)pyrene hydroxylase (BP) activity was determined. The correlation coefficient is given by "r".

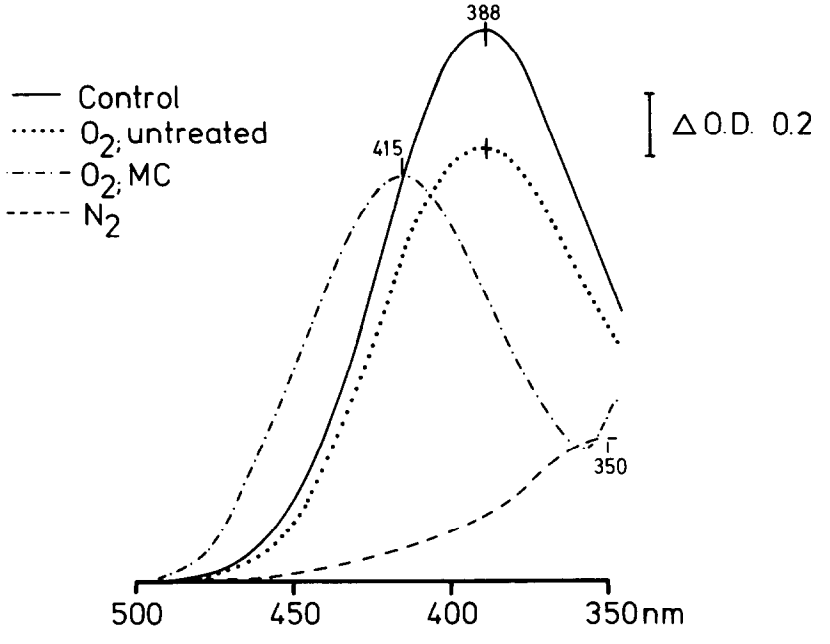

Figure 2: Absorbance spectra of perfusate after rat liver perfusion with nitrofurantoin under various conditions. Nitrofurantoin (0.1 mM) was added to the perfusion medium and the absorbance spectrum was primarily recorded before entering the liver (Control). The other spectra of the perfusate were recorded at the end of a 40 min period of three different perfusion experiments under the following conditions: aerobic perfusion using the liver of an untreated animal (O_2; untreated); anaerobic perfusion without previous induction (N_2); aerobic perfusion after induction with 3-methylcholanthrene (O_2; MC). The spectra were recorded at a pH of 7.8 in the split beam mode using perfusion medium without nitrofurantoin as the reference.

Extraction of the perfusate with nitromethane separates NF from the material with the absorbance maximum near 415 nm. NF is extracted into the organic phase, the metabolite remains almost completely in the aqueous phase exhibiting an intensive yellow color due to the absorbance maximum at 415 nm. The formation of this metabolite in the perfusate is linear with time and can virtually only be detected after MC- or βNF-induction (Table 1). Pretreatment

with MC as well as βNF-but not with PB-leads also *in vivo* to an enhanced formation of a yellow, polar metabolite in the urine after feeding of NF to rats (Table 1).

DISCUSSION

The results indicate that induction with MC or βNF leads to an enhanced oxidative biotransformation of NF in the isolated liver. Simultaneously, a yellow, polar metabolite with an absorbance maximum at 415 nm is formed which is almost exclusively detectable after induction with MC or βNF but not in livers derived from untreated or PB-treated animals. This suggests a new hepatic pathway of NF-metabolism occuring only after induction with inducers of cytochrome "P-448" as indicated also by the strong correlation between hepatic NF-clearance and microsomal benzo(a)pyrene hydroxylase activity serving as a rather induction-specific parameter (Figure 1).

The increase of the urinary excretion of unchanged NF after an oral dose of NF in animals pretreated with MC or βNF also indicates an enhanced biotransformation of NF under *in vivo*-conditions. The enhanced excretion of a yellow, polar metabolite after feeding NF *in vivo* may prove as a suitable *in vivo*-indicator of the cytochrome "P-448"-dependent induction state of the organism.

Previous studies have shown a pathway of nitrofuran derivatives leading to the formation of bright yellow, polar metabolites absorbing near 415 nm which were found in the urine of animals fed the nitrofurans (13, 14). Subsequently, those metabolites could be identified in the urine of rats fed NF as tautomeric mixtures of 4-hydroxy-5-nitrofurancarboxaldehyde derivatives and the corresponding *aci*-nitro form, indicating metabolic hydroxylation of the furan ring (4).

Though the chemical nature of the induction-dependent metabolites in perfusate and urine is not yet known, it appears possible that induction with MC or βNF leads to an enhanced hydroxylation at the furan ring in the 4-position.

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