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CYTOCHROME P-450 AND ETHOXYCOUMARIN-DEETHYLATION IN RAT GASTRIC MICROSOMES: INDUCTION BY 3-METHYLCHOLANTHRENE AND INHIBITION BY CIMETIDINE

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SUMMARY: Gastric microsomal preparations from the rat showed a considerable cytochrome P-450 content, which is inducible by oral dosing of 3-methylcholanthrene. Preparations were able to dealkylate 7-ethoxycoumarin. A typical ligand spectrum by interaction with ferricytochrome P-450 upon addition of the anti-ulcer drug cimetidine to gastric microsomes was observed. Cimetidine also inhibited 7-ethoxycoumarin deethylation. This inhibition was more pronounced in control microsomes than in microsomes from 3-methylcholanthrene pretreated rats.

INTRODUCTION

Knowledge on cytochrome P-450 dependent monooxygenase in the stomach wall is relatively scarce as compared with data on the intestinal mucosa (1, 2, 3). Recently Guengerich and Mason (4) reported low cytochrome P-450 levels in rat stomach and confirmed earlier observations of Wattenberg et al. (5) who demonstrated benzo(α)pyrene hydroxylase activity in the stomach of the rat after oral administration of 1,2-benzanthracene. No deethylation of 7-ethoxycoumarin could be detected.

We have investigated the occurrence of cytochrome P-450 activity in the stomach wall of rats and studied the effect of induction by 3-MC and addition of cimetidine. Cimetidine is extensively used as an anti-ulcer drug (6). Only recently several papers dealing with possible drug-drug interactions in humans involving cimetidine were published. This interaction could be due to inhibition of hepatic cytochrome P-450 depen-

Abbrevations: 3-MC: 3-methylcholanthrene; 7-EC: 7-ethoxycoumarin
DMSO: Dimethylsulfoxide

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dent oxidative reactions in case of warfarin (7), diazepam (8, 9), theophylline (10, 11), chloordiazepoxide (12, 13) and aminopyrine (14) or may be the effect of inhibited gastric acid secretion on drug absorption (15, 16).

A similar inhibition is now reported for gastric cytochrome P-450.

MATERIALS AND METHODS

Adult Male Wistar Rats weighing approximately 250 g (TNO, Zeist) were used. Pretreated animals received a single intragastric injection of 1.1 ml corn-oil or 3-methylcholanthrene (3-MC), 20 mg/kg body weight, in 1.1 ml corn-oil by stomach tube 48 hours before preparation of stomach microsomes.
After anaesthesia with ether, stomachs were excised, prepared free of mucus, rinsed in ice-cold phophate buffer (50 mM and pH 7.4) containing 0.1 mM EDTA, blotted dry and weighed. Pooled stomachs obtained from at least 4 animals were homogenized in 2 volumes of phosphate buffer using an Ultra-Turrax (Janke & Kunkel KG), for 2 x 10 seconds.
The homogenate was centrifuged at 9000 g for 20 min at 4°C. The microsomal fraction was sedimented from the supernatant by 60 min centrifugation at 100,000 g and 4°C. The pellet was resuspended in the same ice-cold buffer (0,5 g stomach/ml) and was used within 3 hours after preparation.

NADP, G6-P (disodiumsalt), G6P-DH (grade I) and 7-ethoxycoumarin were purchased from Boehringer Mannheim. 3-Methylcholanthrene was obtained from Fluka and cimetidine was a gift from S.K. & F.

The O-Dealkylation of 7-ethoxycoumarin was determined according to Greenlee & Poland (17). The incubation medium consisted of phosphate-buffer (50 mM, pH 7.4) containing 0.1 mM EDTA. 7-EC-concentration was varied between 50 and 500 µM. Microsomes obtained from 0.25 g stomach (2.6 - 2.9 mg protein) were used per incubation vessel in a total volume of 3.0 ml. The reaction was started by addition of 0.5 ml 7-EC in water/methanolic solution (final methanol concentration 1.2%). Incubations were stopped after 30 min by addition of 375 µl 15% TCA.

Difference spectra were recorded at 37°C using an Aminco DW-2 UV-Vis spectrophotometer in the split-beam mode. For each determination the stomachs of at least 4 animals were pooled. Microsomal suspensions were equally divided between sample and reference cuvettes and a baseline of zero absorbance was established. Cimetidine, dissolved in dimethylsulfoxide was added to the sample cuvette and an equal amount of dimethylsulfoxide in the reference cuvette.
The concentration of cytochrome P-450 was estimated by means of a dithionite difference spectrum (18) ($E = 100$ mM cm^{-1}) thereby preventing interference by any hemoglobin which might have contaminated the samples.
Protein was determined according to Lowry et al. (19).

RESULTS

Microsomes from the stomach were prepared without adding various protease inhibitors in homogenization. No decrease in cytochrome P-450

Table 1: Cytochrome P-450 content in gastric microsomal preparations in nmoles gr stomach

controls	3-MC pretreated
0.472 (6)[a]	0.568 (9)
0.475 (5)	0.53 (6)
0.480 (4)	
0.480 (6)	
mean: 0.477 ± 0.004 (n = 4)	0.549 ± 0.027* (n = 2)

*Significantly different from controls ($P < 0.025$, Student-t).

[a]The figure in parentheses is the number of stomachs pooled for one experiment. Mean weight per stomach 1.0 ± 0.1 g (n = 35). Protein content varies between 10.6 and 11.5 mg protein 1 g stomach.

content and drug metabolizing activity (7-EC) occurred, when measured during several hours after homogenization. The content of cytochrome P-450 was significantly ($P < 0.025$) higher in microsomes of 3-MC pretreated rats gastric microsomal preparations than in controls (Table 1). Also microsomes from stomachs of 3-MC pretreated rats showed a marked increase (8-fold, Table 2) in 7-EC O-deethylation as compared with oil treated rats. So mono-oxygenase induction was greater than cytochrome P-450 enhancement after 3-MC treatment.

The Lineweaver-Burk plots of the inhibition of the O-dealkylation of 7-EC (fig. 1.a, b) show a competitive inhibition by cimetidine (Table 2) for control and 3-MC pretreated rats. Calculation, however, of the inhibition constant, K_i, clearly indicated a difference between control (K_i = 0.64 mM) and 3-MC pretreated rats (K_i = 1.25 mM). For K_m and V_{max} values see Table 2.

Difference spectra produced from titration with cimetidine of the gastric microsomal suspension of control rats and rats pretreated

Table 2: Influence of pretreatment with 3-MC and/or the presence of cimetidine on the O-dealkylation of 7-ethoxycoumarin in vitro by gastric microsomal preparations

	K_m (mM)	V_{max} (pmol·g stomach^{-1}·30 min^{-1})
control	0.335	103
control + cimetidine (500 µM)	1.94	134
3-MC	0.156	860
3-MC + cimetidine (500 µM)	0.217	855

with 3-MC showed a typical ligand spectrum with a peak at 430 nm and a trough at about 397 nm (see Fig. 2). The magnitude of the spectral change is somewhat lower for 3-MC pretreated rats [$\Delta A_{430-397}$ = 0.014 nmol P-450^{-1}·ml^{-1}] than in controls [$\Delta A_{430-397}$ = 0.019 nmol P-450^{-1}·ml^{-1}]. These values are in the same order of magnitude as the absorption found by Rendic et al. (20) using liver microsomes of 3-MC pretreated rats in

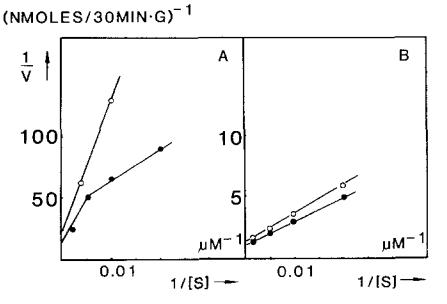

Fig. 1 Lineweaver-Burk plots of the inhibition of the O-dealkylation of 7-ethoxycoumarin.
● : without addition of cimetidine
○ : cimetidine added, final concentration 500 µM.
A. Dealkylation of 7-EC by gastric microsomal preparations from control rats.
B. Id., for 3-MC pretreated rats.

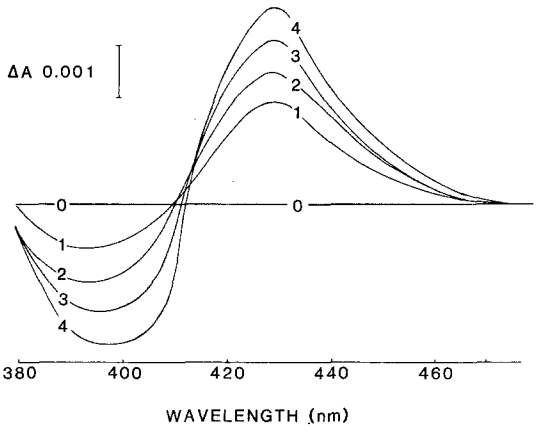

Fig. 2 Difference spectra from titration of gastric microsomes of 3-MC pretreated rats with cimetidine. After establishing the baseline (0), the contents of the sample cuvette were titrated with cimetidine (1-4 are respectively 0.33, 0.67, 0.9 and 1.2 mM) dissolved in DMSO. An equal volume of DMSO was added to the reference cuvette. The cuvettes contained 5.4 mg protein/ml.

the presence of cimetidine (700 μM) [$\Delta A_{429-392}$ = 0.026 nmol P-450^{-1}·ml^{-1} for pretreated and 0.0013 for controls].

DISCUSSION

We were able to determine reproducible values for cytochrome P-450 per g organ weight or per mg protein in rat stomach. In contrast to Guengerich and Mason (4) we also demonstrated a significant induction by dosing orally 3-MC (20 mg/kg, 48 hours before preparation), of cytochrome P-450 and 7-EC-O-deethylase in microsomal preparations of rat stomach. Guengerich et al. could not detect a significant 7-EC-O-deethylase activity probably because of the relatively low cytochrome P-450 content in the incubate as well as the short incubation time. Just as with liver preparations (14, 20) the interaction of cimetidine with gastric cytochrome P-450 gives rise to a characteristic ligand spectrum and inhibits the O-dealkylation of 7-ethoxycoumarin competitively. Pelkonen (14) already mentioned a discrepancy between the inhibitory properties of cimetidine on benzpyrene hydroxylase activity and spectral interactions.

The maximal spectral change calculated per unit of cytochrome P-450 was largest with microsomes from 3-MC pretreated rats, whereas benzpyrene hydroxylation was hardly inhibited by cimetidine (non-competitive). In this study another P-448 mediated reaction, the O-dealkylation of 7-EC, showed similar results with gastric microsomal preparations. A relatively stronger inhibition by cimetidine in control microsomes than in preparations from 3-MC pretreated rats was observed. The magnitude of the spectral change induced by cimetidine however is somewhat lower in induced rats, although not significantly so.

These findings indicate once more that cimetidine is an inhibitor of cytochrome P-450 mediated reactions, even at the site of its pharmacological action, the stomach. The exact mechanism of the inhibition, however, is still unknown.

Because of the inhibitory action of cimetidine on cytochrome P-448 mediated reactions, this drug may play a protective role preventing activation of precarcinogens in the digestive tract.

References

1. Hoensch, H.P., Hutt, R., Hartmann, F. (1979) Env. Hlth. Perspectives, 33, 71-78
2. Chhabra, R.S. (1979) Env. Hlth. Perspectives, 33, 61-69
3. Wollenberg, P., Ullrich, V. (1980) in "Extrahepatic Metabolism of drugs and other foreign compounds", 1980, 267-276, ed. Th.E. Gram, MTP Press Ltd.
4. Guengerich, F.P., Mason, P.S. (1979) Mol. Pharmacol., 15, 154-164
5. Wattenberg, L.W., Leong, J.L., Strand, P.J. (1962) Cancer Res., 22, 1120-1125
6. Burland, W.L., Geladle, R.I., Mills, J.G., Sharpe, P.C., Wells, A.L. (1977) in "Cimetidine. Proceedings of the second Internation Symposium on Histamine H_2-receptor Antagonist", 67-75, Excerpta Medica, Amsterdam
7. Serlin, M.G., Sibbon, R.G., Mossman, S., Breckinridge, A.M., Williams, J.R.B., Atwood, J.L., Willoughbt, J.M.T. (1979) Lancet ii, 317-319
8. Klotz, U., Antilla, V.J., Reimann, I. (1979) Lancet ii, 699
9. Klotz, U., Reimann, I. (1980) New Engl. J. Med., 302, 1012-1014
10. Weinberger, M.M., Smith, G., Milavetz, G., Hendeles, L. (1981) The New Engl. J. Med., 304, 672
11. Van Boxtel, C.J., Oosterhuis, B., van Zandwijk, N., Braat, M.C.P. (1981) Proc. 22nd Dutch Fed. Meeting 1981, Nijmegen, Fed. Med. Scient. Soc.
12. Desmond, P.V., Patwardhan, R.V., Schenker, S., Speeg, K.V. Jr. (1980) Ann. intern. Med., 93, 266-268

13. Ruffalo, R.L., Thompson, J.F. (1980) New Engl. J. Med., 303, 753-754
14. Pelkonen, O., Puurunen, J. (1980) Biochem. Pharm., 29, 3075-3080
15. Cole, J.J., Charles, B.G., Ravenscroft, P.J. (1980) Lancet ii, 536
16. McCloy, R.F., Baron, J.H. (1981) Lancet, march 14, 609
17. Greenlee, W.F., Poland, A. (1978) J. Pharm. Exp. Ther., 205, 596-505
18. Estabrook, R.W., Peterson, J.A., Baron, J., Hildebrandt, A., in "Methods in Pharmacology" (ed. C.F. Chignell) Vol. 2, 303-350, Appleton-Century-Crofts, New York
19. Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J. (1951) J. Biol. Chem., 193, 265-275
20. Rendic, S., Sunjic, V., Toso, R., Kajfez, F. (1979) Xenobiotica, 9, 555-564