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Inhibition of the human heart and lung NADPH-cytochrome P-450 reductase activity by the presence of 4-methylpyrazole

4-Methylpyrazole (4-MP) is a drug that inhibits alcohol dehydrogenase, the enzyme which catalyzes oxidation of alcohols. Some of the P450 isoenzymes, including CYP2E1, are also inhibited by 4-MP (3, 4, 6, 7). CYPs isoenzymes catalyze the oxidation of substracts in the presence of O_2 and NADPH, resulting in the generation of specific product, NADP and H_2O. The electron transfer from NADPH to alcohol goes via NADPH-cytochrome P-450 reductase, the key enzyme in this mechanism. NADPH-cytochrome P-450 reductase is involved in the metabolism of endogenous compounds, such as some steroid hormones, and plays a crucial role in the first phase of detoxification of various xenobiotics.

The aim of this study was to evaluate the effect of 4-MP on NADPH-cytochrome P-450 reductase activity in human liver, lungs and heart microsomes.

MATERIAL AND METHODS

This experiment was designed according to international guidelines and the national law.

Chemicals. All chemicals obtained from commercial sources were of the highest purity and were used without further purification. 4-Methylpyrazole and NADPH were purchased from Sigma Chemical Co. (St. Louis, USA). Cytochrome c (oxidised form) was ordered in Fluka Chemica, Switzerland.

Tissue preparation. Seven samples of human male livers, lungs and hearts were collected 24h after death and stored at -70°C until analysed. The causes of death were traffic accidents. Immediately after defrosting samples of human tissues were rinsed with buffer (150 mM KCl, 50 mM Tris, pH 7.4), minced with scissors, and homogenized on ice in a motor-driven Potter-Elvehjem homogenizer with three volumes of buffer. The crude homogenate was centrifuged at 8,750 g for 15 min at 4°C. Microsomes were sedimented from the 8,750 g supernatant by centrifugation at 165,000 g for 38 min at 4°C. After two washing steps, microsomes were dispersed in homogenizing buffer to provide a protein concentration of approximately 30 mg/ml.

Enzyme assay. The activity of NADPH-cytochrome P-450 reductase (E.C. 1.6.2.4.) was evaluated by measuring the rate of cytochrome c reduction at 550 nm (2). Microsomes were incubated

with 0.01mM, 0.10mM and 1.00mM (final concentration) of 4-methylpyrazole in 33mM phosphate buffer pH 7.7 in the presence of 1 mM KCN. Cytochrome c was used (0.025mM) as an electron acceptor. Reaction was started by the addition of NADPH (0.1 mM) after 5min incubation at 25°C. The activity of NADPH-cytochrome P-450 reductase was monitored, during 2 min after NADPH addition every 30 seconds using Shimadzu UV-160A spectrophotometer. In control samples 4-methylpyrazole was replaced with a buffer. Milimolar extinction coefficient was used ($e=0.021 mM^{-1}cm^{-1}$) for calculation and enzyme activity was expressed as nmoles of reduced cytochrome c/min/mg microsomal protein. The content of microsomal protein was measured using Folin-Ciocalteu reagent. Bovine serum albumin was used as standard.

Statistical analysis. The obtained data were analyzed using STATISTICA 5.0. The homogeneity was examined using the Shapiro-Wilk's test. In order to compare the differences between the experimental and control groups The Mann-Whitney's U test was used for statistical analysis. An á=0.05 ($p<0.05$) was considered significant.

RESULTS

Microsomes were incubated with 0.01mM, 0.10mM and 1.00mM of 4-methylpyrazole. The highest concentration of 4-MP significantly decreased lung P450 reductase activity by about 30.48%. A similar effect was observed when heart microsomes were incubated with 4MP at final concentration of 0.10 mM and 1.00 mM. The decrease in the tested enzyme activity was about 44.71% and 39.68%, respectively The activity of P450 reductase was statistically unchanged when liver microsomes were incubated with all tested concentrations of 4-MP.

Table 1. NADPH- cytochrome P-450 reductase activity in human tissue microsomes incubated with 4-metylpyrazole [nM cyt. c/ min/ mg microsomes protein]. Results are mean (M) ± SD for seven observations with comparison to the control group. P-450 reductase activity was assayed spectrophotometrically at 25° C and pH 7.7, *in vitro*

	Group	Min	Max	M ± SD	Me	Δ	Δ%	P
Liver	Control	121.00	192.00	161.29 ± 24.55	167.00			
	0.01mM	147.00	208.00	174.78 ± 28.65	181.00	13.49	8.36	ns
	0.10mM	113.00	189.00	154.33 ± 24.48	156.00	-6.96	-4.32	ns
	1.00mM	123.00	178.00	156.82 ± 23.93	152.00	-4.47	-2.77	ns
Lung	Control	138.00	213.00	168.86 ± 27.30	162.00			
	0.01mM	144.00	226.00	172.73 ± 26.68	170.00	3.87	2.29	ns
	0.10mM	106.00	175.00	159.49 ± 22.54	157.00	-9.37	-5.55	ns
	1.00mM	86.00	136.00	117.39 ± 17.38	114.00	-51.47	-30.48	< 0,05
Heart	Control	8.00	22.00	13.71 ± 4.64	14.00			
	0.01mM	8.00	19.00	12.49 ± 3.82	13.00	-1.22	-8.90	ns
	0.10mM	6.00	13.00	7.58 ± 4.91	9.00	-6.13	-44.71	< 0,01
	1.00mM	5.00	11.00	8.27 ± 4.17	8.00	-5.44	-39.68	< 0,05

$\Delta = M_{control} - M_{samples}$; $\Delta\ \% = (M_{samples} \times 100\% / M_{control}) - 100\%$

DISCUSSION

The NADPH-cytochrome P-450 reductase (P-450 reductase) is the main component of electron transport chain in microsomes. The microsomal electron transport system is involved in the metabolism of endogenous compounds, such as some steroid hormones, and plays a crucial role in the first phase

of detoxification of many xenobiotics. It should be stressed, however, that in some cases metabolised xenobiotics are more toxic than parent compounds. P-450 reductase is responsible e.g for the toxic effect of the cytostatic doxorubicin and the herbicide paraquat. The enzyme carries one electron from a drug to O_2 forming superoxide radical, thus doxorubicin in the heart and paraquat, especially in the lung, trigger free radical transformation pathways (1, 5) therefore inhibitors of P-450 reductase activity may be very useful in clinical practice. Hence, 4-Metylpyrazole (4-MP) seems to be an effective inhibitor of alcohol dehydrogenase and also potent inhibitor P450 CYP2E1 (7). The effect of 4-MP on P-450 reductase activity in human heart, liver and lungs was evaluated in this study.

As shown in table I the activity of P450 reductase was statistically unchanged when liver microsomes were incubated with all tested concentrations of 4-MP. However, it is impossible to state definitely that 4-MP could not affect the xenobiotic metabolism via P-450 reductase. Further investigations are needed to elucidate if 4-MP may suppress or induce a single gene (pter-q22, located on chromosome 7) encoding P450 reductase (9). The highest concentration of 4-MP decreased lung P450 reductase activity by about 30.48%. A similar effect was observed when heart microsomes were incubated with 4-MP at final concentration of 0.10 mM and 1.00 mM. The decrease in the tested enzyme activity was about 44.71% and 39.68%, respectively. It should be explained why the same concentration of the tested compounds leads to different effects (Ä) of P-450 reductase in microsomes obtained from different tissues. Although there is no information concerning tissue specificity of P-450 reductase expression, it seems that the expression of this enzyme is different in the tested tissues. The observation of physiological activity in liver, heart and lung (in controls – buffer was used instead of a tested compound) may confirm this theory. Moreover, Hall et al. (8) found specific cellular localization of P-450 reductase in human tissues. It is possible, that such differences may be partially responsible for the specific toxic localisation of paraquat in lung, and adraiamycin in heart.

CONCLUSIONS

1. It seems that inhibitory effect of the 4-MP on lung and heart P450 reductase activity may have a desirable effect during doxorubicin therapy and paraquat toxicity.

2. 4-Metylpyrazole does not change P450 reductase activity in liver and thus cannot significantly affect the liver alcohol metabolism by this pathway. However, further investigation is needed to elucidate if 4-MP may suppress or induce a single gene encoding P450 reductase.

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SUMMARY

The aim of the study was to evaluate the effect 4-metylpyrazole on human heart, lung and liver NADPH-cytochrome P-450 reductase activity. The preliminary study was carried out on tissue sections obtained from 7 males. No microscopic symptoms of autolysis or other pathomorphological changes were found in the examined organ samples during histological examination. The activity of NADPH-cytochrome P-450 reductase [E.C. 1.6.2.4.] was evaluated by measuring the rate of cytochrome c reduction at 550 nm. Microsomes were incubated with 0.01mM, 0.10mM and 1.00mM of 4-methylpyrazole. In control samples 4-methylpyrazole was replaced with a buffer. The highest concentration of 4-MP significantly decreased lung P450 reductase activity. A similar effect was observed when heart microsomes were incubated with 4MP at final concentration of 0.10 mM and 1.00 mM. The activity of P450 reductase was statistically unchanged when liver microsomes were incubated with all tested concentration of 4-MP. It seems that the inhibitory effect of the 4-MP on lung and heart P450 reductase activity may have a desirable effect during doxorubicin therapy and paraquat toxicity.

Hamowanie aktywności reduktazy cytochromu P-450 w ludzkich mikrosomach serca i płuc przez 4-metylopirazol

Celem pracy była ocena działania 4-metylopirazolu na aktywność reduktazy cytochromu P-450 w mikrosomach, wyizolowanych od ludzi z mięśnia serca, płuc oraz wątroby. Badania *in vitro* zostały przeprowadzone na skrawkach tkanek, pochodzących od siedmiu osób płci męskiej zmarłych śmiercią gwałtowną, w których we wstępnych badaniach nie stwierdzono zmian histologicznych. Aktywność reduktazy cytochromu P-450 była oznaczana poprzez pomiar stopnia redukcji cytochromu c przy długości fali 550 nm. Początkowo w mikrosomach wyizolowanych z serca, płuc oraz wątroby mierzono aktywność fizjologiczną reduktazy cytochromu P-450. Następnie w tych samych warunkach przeprowadzano pomiar aktywności badanego enzymu w obecności trzech różnych stężeń 4-metylopirazolu: 00.1 mM; 0.10 mM i 1.00 mM. Stwierdzono, że 4-metylopirazol w stężeniu 1.00 mM i 0.10 mM hamował aktywność badanego enzymu w mikrosomach serca, a zmiana ta była istotna statystycznie. W mikrosomach wyizolowanych z płuca obserwowano również istotne statystycznie hamowanie aktywności enzymu przy najwyższym stężeniu 4-metylopirazolu. Nie stwierdzono istotnego wpływu 4-metylopirazolu na aktywność reduktazy cytochromu P-450 wyizolowanej z mikrosomów wątroby. W świetle przeprowadzonych badań można oczekiwać, że 4-metylopirazol może wywierać pożądane działanie w kardiotoksyczności antracyklin oraz toksycznym uszkodzeniu płuc, w zatruciach parakwatem.