The influence of single application of paracetamol and/or N-acetylcysteine on rats subchronic exposed to trichloroethylene vapours. I. Effect on hepatic moonooxygenase system dependent of cytochrome P450

Wpływ pojedynczej dawki paracetamolu i/lub N-acetylocysteiny na szczury przewlekle eksponowane na trichloroetylen. I. Wpływ na wątrobowy układ monooksygenaz zależnych od cytochromu P450

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(c) *Collection of material for research*
(d) *Laboratory tests*
(e) *Statistics*
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Abstract

Background: There is a number of factors which potentially affect occurrence of toxic change in liver after overdosing of paracetamol. Hepatic metabolism of trichloroethylene has primary impact on hepatotoxic effect of this solvent. This means that the combined exposure to these xenobiotics can be particularly harmful for human. The influence of N-acetylcysteine (NAC) as a protective factor after paracetamol intoxication was studies.

Materials and method: Tests were carried out on rats which were treated with trichloroethylene, paracetamol and/or N-acetylcysteine. In the hepatic microsomal fraction activity of the components of cytochrome P450-dependent monooxygenases was determined

Results: Paracetamol slightly stimulated cytochrome P450 having no effect on reductase activity cooperating with it. Cytochrome b_5 and its reductase were inhibited by this compound. Trichloroethylene was the inhibitor of compounds of II microsomal electron transport chain. N-acetylcysteine inhibited activity of reductase of NADH-cytochrome b_5.

Conclusions: Tested doses of the xenobiotics influenced on II microsomal electron transport chain. Protective influence of N-acetylcysteine was better if this compound was applied 2 hours after exposure on xenobiotics.

Keywords: cytochrome P450, liver, trichloroethylene, paracetamol, N-acetylcysteine

Streszczenie

Wstęp: Istnieje szereg czynników, które potencjalnie wpływają na ryzyko wystąpienia zmian toksycznych w wątrobie po przedawkowaniu paracetamolu. Wątrobowy metabolizm trichloroetylenu ma pierwszorzędny wpływ na hepatotoksyczny efekt tego rozpuszczalnika. Oznacza to, że narażenie łączne na te ksenobiotyki może być szczególnie szkodliwe dla człowieka. Oceniono wpływ N-acetylocysteiny (NAC) jako czynnika osłaniającego po zatruciu paracetamolem.

Nadesłano: 28.02.2012
Zatwierdzono do druku: 26.03.2012

Materiał i metody: Badania wykonano na szczurach, które traktowano trichloroetylenem, paracetamolem i/lub N-acetylocysteiną. We frakcji mikrosomalnej wątroby oznaczano aktywność składników monooksygenaz zależnych od cytochromu P450.

Wyniki: Paracetamol lekko stymulował cytochrom P450 nie mając wpływu na aktywność reduktazy współpracującej z nim. Cytochrom b_5 i jego reduktaza były hamowane przez ten związek. Trichloroetylen był inhibitorem składników II mikrosomalnego łańcucha transportu elektronów. N-acetylocysteina hamowała aktywność reduktazy NADH-cytochrom b_5.

Wnioski: Badane dawki ocenianych ksenobiotyków swój wpływ ujawniały raczej na składniki II mikrosomalnego łańcucha transportu elektronów. Ochronny wpływ N-acetylocysteiny był wyraźniejszy, jeśli podano ten związek 2 godziny po zakończeniu ekspozycji na badane ksenobiotyki.

Słowa kluczowe: cytochrom P450, wątroba, trichloroetylen, paracetamol, N-acetylocysteina

Introduction

In the liver, cytochrome P450-related monooxygenase system is responsible for endogenous and exogenous substances biotransformation, the activity of which depends on numerous environmental factors [1–3]. Reflections concerning metabolic alterations should be combined with the analysis of other important cellular processes of hepatocyte. Impairment of the function of these processes leads to cell feeding disturbances [3] which can ultimately affect detoxication reactions [4].

Paracetamol (acetaminofen, APAP), is both an analgesic and antipyretic which in therapeutic doses is safe [5]. When overdosed, it can damage the liver [6] through a toxic metabolite, N-acetyl-p-benzochinonoimine [7]. At high doses of APAP, detoxication ability of the liver is saturated and there is accumulation of intermediate products.

Paracetamol overdose causes fatal liver toxicity, first time reported in people in the mid 1960s. Since then, paracetamol poisoning has been the most common cause of a severe liver failure in many countries. Paracetamol poisoning may result from evident overdose (when the medication is taken in high dose, usually within 1 hour), or as an overdose taken in a longer period.

There are several factors which potentially affect the development of liver toxicity following paracetamol overdose. They may be divided into the following categories: dose of paracetamol taken; time between dose taking and start of treatment, e. g. with N-acetylcysteine; increased paracetamol metabolism into NAPQI due to proper CYP isoforms induction; and decreased level of endogenous glutathione in hepatocytes.

Trichloroethylene (TRI) is an organic solvent used in industry and a common atmospheric pollutant. In rats, TRI causes a minor liver damage resulting in an increase of plasma marker activity and histological necrotic hepatocytes in zone III of liver acini [8]. TRI metabolism has a primary impact upon hepatotoxic effect of this solvent. P450 isoforms are the key enzymes in the first stage of this metabolic compound metabolism i. e., formation of chloral hydrate, which is the stage limiting reaction rate and TRI metabolism and is significant in TRI hepatotoxicity and carcinogenicity [9–11]. It was shown in animals treated with a variety of xenobiotics metabolized by cytochrome P450-related hepatic monooxygenases (so-called I microsomal electron transport chain), that increased TRI transformation into metabolites secreted with urine is simultaneous with an increased hepatic level of P450. We must remember, that in risk situations the cells switch on one more enzymatic system. This system is working with cytochrome b_5, which makes II microsomal electron transport chain. Cooperation between the two chains provide optimal process of detoxification of xenobiotics for cell.

The aim of this study was to examine the effect of paracetamol and/or trichloroethylene on activity and content of hepatic cytochrome P450-dependent monooxygenase system. Very important are factors evaluated during estimation of the toxicity after paracetamol poisoning and their affect on decision about their administration. Therefore, in this study we evaluate the effect of N-acetylcysteine (NAC) as a protective factor on enzymatic system of liver after paracetamol poisoning.

Materials and methods

Animals

The examinations were conducted on male Wistar rats with body mass 280–300 g. The animals were kept separately in plastic cages throughout the examination in controlled culture conditions with constant air humidity (60%), constant temperature (22 ± 2°C) and 12 hour cycle day/night. The animals were fed on Murigan type standard granulated fodder, with unlimited water access.

This research was approved by the Local Bioethics Committee of The Medical University in Poznań.

Experiment outline

The animals were divided into groups, 6 in each. They were administered xenobiotics separately and collectively according to the following regimen:

1. The control group
2. APAP – 250 mg/kg b.m.
3. TRI – 50 mg/m^3
4. NAC – 150 mg/kg b.m.
5. TRI 50 mg/m^3 + NAC (0 h) 150 mg/kg b.m.
6. TRI 50 mg/m^3 + NAC (2 h) 150 mg/kg b.m.
7. APAP – 250 mg/kg m.c. + TRI 50 mg/m^3
8. APAP – 250 mg/kg b.m. + NAC (0 h) 150 mg/kg b.m.
9. APAP – 250 mg/kg b.m. + NAC (2 h) 150 mg/kg b.m.
10. APAP – 250 mg/kg b.m. + TRI 50 mg/m^3 + NAC (0 h) 150 mg/kg b.m.
11. APAP – 250 mg/kg b.m. + TRI 50 mg/m^3 + NAC (2 h) 150 mg/kg b.m.

The control group were the animals not exposed to the mentioned xenobiotics. Experimental group animals were exposed to TRI vapours through inhalation route in the dynamic toxicological chamber in concentration 50 mg/m^3 of air for the following 7 days, 6 hours daily. Exposure to TRI lasted between 9.00 and 15.00. On the last day of exposure, at 9.00 they were administered APAP by stomach tube. NAC was administered along with examined xenobiotics right after the exposure (0 h) or 2 hours following their application (2 h).

Isolation of liver microsomes

Rats were sacrificed between 8.30 and 9.30 a.m. to avoid circadian fluctuations in the activity of the cytochrome P450-dependent monooxygenase system [12, 13]. Liver samples were placed in ice-cold physiological saline. The microsomal fraction was isolated by the method of Dallner [14].

The activity of the monooxygenase system were measured at the following time points: 12-, 24-, 48- and 120 hours after treatment.

Monooxygenase components

Cytochrome P450 content and cytochrome b5 content were determined by the method of Estabrook and Werringloer [15]. Their levels were calculated with the use of millimolar absorption coefficients (91 $mM^{-1}cm^{-1}$ and 185 $mM^{-1}cm^{-1}$, respectively) and expressed in nanomoles of a cytochrome per 1 milligram of microsomal protein.

The activities of NADPH-cytochrome P450 reductase and NADH-cytochrome b_5 reductase were evaluated by measuring the speed of cytochrome c reduction at 550 nm (Hodges and Leonard [16]). The concentration of cytochrome c were calculated with the use of the molar extinction coefficient 18.5 $mM^{-1}cm^{-1}$ [16]. Both the reductase activities were expressed in micromoles of cytochrome c per 1 min/mg protein.

Determination of protein

Microsomal protein concentration was determined by the method of Lowry et al. [17] with bovine albumin as a standard.

Statistical analysis

Characteristics of the examined parameters was presented in a form of arithmetic mean as a measure of central tendency and standard deviation as a measure of variability. Distribution normality of the examined parameters was verified with Kolmogorov-Smirnov accordance tests and Shapiro-Wilk test and visually evaluated by histograms. Distributions close to normal were considered those for which significance level as a result of testing was greater than 0.05 in both tests, and whose histogram shape was symmetrical. Distributions of all examined parameters were considered standard.

To estimate the effect of the examined xenobiotics on the level of cytochrome 450-dependent hepatic monooxygenases system (in subsequent time periods of 12-, 24-, and 120 hours) ANOVA variance analysis was used. Assumptions concerning variance homogeneity in this analysis were verified by means of Levene's test. As post-hoc test (to compare individual means with controls) in this ANOVA analysis, Dunnett's multiple comparison test was performed. The results were considered statistically significant with $p < 0.05$ and were marked in tables with the symbol "*". Statistical analyses were conducted using a professional set of STATISTICA PL statistical procedures, version 8,0.

Results

Treatment with paracetamol did not lead to a change in P450 cytochrome content during the first 24 hours of experiment, then it distinctly increased after 48 hour (Table I). Since then, the level of this hemoprotein was slightly falling, and after 5 days virtually reached the value of the control group. The activity of NADPH-cytochrome P450 reductase kept the control level and did not change till the completion of the experiment.

Cytochrome b_5 negatively reacted to paracetamol in the first stage of observation. Between 12 to 48 hours there was a tendency to slow inhibition. On the day 5 this cytochrome regained the control level. Reductase cooperating with cytochrome b_5 responded differently to this xenobiotic. After 12 hours its activity decreased, showing a tendency to slow inhibition. This condition altered into activity inhibition and lasted till the completion of the experiment.

Table I. Effect of TRI, APAP or NAC on cytochrome P450-dependent moonooxygenase system in rat liver
Tabela I. Wpływ TRI, APAP lub NAC na poziom wątrobowego układu monooksygenaz zależnych od cytochromu P450 u szczura

Protein	Xenobiotic	Time after exposure [in hours]				
		The control	12	24	48	120
Cytochrome P450	APAP	0.83 ± 0.09	1.04 ± 0.11	1.01 ± 0.12	1.16 ± 0.04*	0.95 ± 0.03
	TRI		0.89 ± 0.06	0.88 ± 0.03	0.93 ± 0.03	0.90 ± 0.05
	NAC		0.82 ± 0.04	0.79 ± 0.01*	0.80 ± 0.05	0.87 ± 0.05
	APAP + TRI		0.87 ± 0.0321	0.90 ± 0.07	0.91 ± 0.06	0.95 ± 0.10
NADPH-cytochrome P450 reductase	APAP	0.09 ± 0.009	0.09 ± 0.009	0.10 ± 0.009	0.10 ± 0.013	0.09 ± 0.009
	TRI		0.11 ± 0.008	0.10 ± 0.004	0.11 ± 0.005*	0.10 ± 0.006
	NAC		0.08 ± 0.007	0.09 ± 0.003	0.09 ± 0.005	0.09 ± 0.006
	APAP + TRI		0.10 ± 0.007	0.11 ± 0.005*	0.12 ± 0.008*	0.12 ± 0.008*
Cytochrome b_5	APAP	0.76 ± 0.04	0.68 ± 0.03*	0.70 ± 0.03	0.71 ± 0.03	0.80 ± 0.02
	TRI		0.78 ± 0.04	0.72 ± 0.02	0.70 ± 0.02*	0.71 ± 0.04
	NAC		0.68 ± 0.03	0.71 ± 0.03	0.72 ± 0.06	0.72 ± 0.05
	APAP + TRI		0.77 ± 0.05	0.74 ± 0.03	0.71 ± 0.02	0.75 ± 0.06
NADH-cytochrome b_5 reductase	APAP	0.82 ± 0.03	0.78 ± 0.02	0.75 ± 0.03*	0.70 ± 0.01*	0.75 ± 0.03*
	TRI		0.74 ± 0.03*	0.69 ± 0.02*	0.70 ± 0.04*	0.68 ± 0.04*
	NAC		0.70 ± 0.06*	0.64 ± 0.03*	0.61 ± 0.02*	0.73 ± 0.05*
	APAP + TRI		0.79 ± 0.08	0.80 ± 0.03	0.82 ± 0.05	0.87 ± 0.03

Treatment with trichloroethylene did not change cytochrome P450 content from the completion of experiment till the end of day 5 of observation (Table I). Reductase cooperating with this cytochrome in the experiment in the first 24 hours of the experiment showed a tendency to increase its activity and enhanced its activity after 48 hours (120% control value), which was maintained almost to 120 hour.

In rats treated with TRI the level of cytochrome b_5 in first 24 hours was comparable to control, but in remaining observation period was below the control group value, revealing its minimal concentration in 48 hour. NADH-cytochrome b_5 reductase responded to TRI in a different manner than cytochrome b_5. Throughout the experiment, activity inhibition of that enzyme was noted, on the level 80-85% of control value.

Administration of N-acetylcysteine resulted in P450 cytochrome level stability, ranging 80–95% of control value (Table I). NAC did not affect NADPH-cytochrome P450 reductase activity.

N-acetylcysteine did not affect cytochrome b_5 content. However, while cytochrome b_5 showed levels slightly below or similar to control throughout the experiment, NADH-cytochrome b_5 reductase exhibited activity definitely below the control level throughout the experiment. After 48 hours inhibition of this reductase was distinct and reached 75% of control value.

Combined exposure to paracetamol and trichloroethylene had no effect upon cytochrome P450 level (Table I). In this experimental line, NADPH-cytochrome P450 reductase activity after 12 hours began to increase, and after 24 hours, 135% stimulation of this activity was shown, lasting till the day 5 of experiment.

Following such treatment, the constituents of II microsomal electron transport chain remained on control level, till completion of experiment.

Simultaneous combined administration of paracetamol and N-acetylcysteine did not affect the level of cytochrome P450 (Table II). Since hour 12 till the completion of the experiment this hemoprotein

was reaching control group level. The activity of NADPH-cytochrome P450 reductase was at control level, and only after 5 days of experiment its activity was slightly stimulated.

Cytochrome b_5 in this research line after 12 hours had a tendency to decrease its level, down to approximately 85% of control value after 5 days. Reductase cooperating with this cytochrome in the first period had activity level close to control, which, since hour 48 was decreasing down to 85% on day 5.

If N-acetylcysteine was administered 2 hours following paracetamol treatment, there was no effect of such treatment on the cytochrome P450 level throughout experiment. Activity of NADPH-cytochrome P450 reductase in these conditions initially retained control level, but 24 hours after completion of experiment its activity tended to increase, exceeding 125% of control value.

Stable cytochrome b_5 control level in this line was observed till 24 hour and a weak stimulation lasting till the end of experiment was noted. In this research there was no effect of NADH-cytochrome b_5 reductase.

Table II. Combined effect of APAP or NAC on cytochrome P450-dependent moonooxygenase system in rat liver
Tabela II. Łączny wpływ APAP i NAC na poziom wątrobowego układu monooksygenaz zależnych od cytochromu P450 u szczura

Protein	Time treatment of NAC	Time after exposure [in hours]				
		The control	12	24	48	120
Cytochrome P450	[0 h]	0.83 ± 0.09	0.82 ± 0.03	0.90 ± 0.0500	0.86 ± 0.05	0.93 ± 0.04
	[2 h]		0.92 ± 0.04	0.96 ± 0.05	0.91 ± 0.02	0.97 ± 0.03
NADPH-cytochrome P450 reductase	[0 h]	0.09 ± 0.009	0.09 ± 0.004	0.10 ± 0.007	0.10 ± 0.003	0.11 ± 0.007*
	[2 h]		0.09 ± 0.007	0.10 ± 0.008	0.11 ± 0.005*	0.11 ± 0.003*
Cytochrome b_5	[0 h]	0.76 ± 0.04	0.80 ± 0.04	0.71 ± 0.04	0.71 ± 0.04	0.66 ± 0.05*
	[2 h]		0.74 ± 0.04	0.77 ± 0.06	0.83 ± 0.04	0.83 ± 0.07*
NADH-cytochrome b_5 reductase	[0 h]	0.82 ± 0.03	0.82 ± 0.05	0.80 ± 0.04	0.75 ± 0.04*	0.70 ± 0.03*
	[2 h]		0.67 ± 0.08	0.77 ± 0.09	0.76 ± 0.06	0.76 ± 0.05

If animals were exposed to combined TRI and NAC (Table III), no significant effect of such exposure was found on cytochrome P450 level. NADPH-cytochrome P450 reductase in the conditions throughout the experiment, exhibited activity close to control, however, by the end of the experiment this activity was found to decrease.

Such treatment of rats had features of a negative effect on cytochrome b_5 content. In these conditions, reductase cooperating with cytochrome b_5, acted similarly as cytochrome b_5 itself.

Administration of N-acetylcysteine 2 hours after TRI, compared with previous experiment line had no significant effect on cytochrome P450 content. Only a tendency to exceed control level was observed, especially by the end of experiment. Similar conclusions apply to NADPH-cytochrome P450 reductase.

Delayed administration of N-acetylcysteine had a negative effect on cytochrome b_5 level (decrease to approximately 85% control value in 24 and 48 hours of experiment duration). This exposure had no effect on NADH-cytochrome b_5 reductase activity. In all experiment stages this enzyme's activity was on control level.

Exposure to simultaneous administration of the three examined xenobiotics had no effect on cytochrome P450 level throughout the experiment (Table IV). Reductase cooperating with this cytochrome did not change its activity in the first 24 hours. After that time, the activity of that enzyme increased. Its elevated level was still observed 5 days after the experiment's completion.

In these experiment conditions, cytochrome b_5 was inhibited, especially in 12-, 24- and 48 hour. NADH-cytochrome b_5 reductase, following simul-

taneous treatment with the three examined xenobiotics, did not change its activity.

If N-acetylcysteine was administered 2 hours after treatment with paracetamol and TRI, cytochrome P450 levels were comparable with control since 24 hours of experiment. NADPH-cytochrome P450 reductase activity exhibited control levels throughout the experiment.

Similarly as in the previous stage of the experiment, cytochrome b_5 level had a small tendency to decrease, however, it was close to control. By contrast, NADH-cytochrome b_5 reductase activity was slightly increased, particularly in 24- and 48 hour of the experiment.

Table III. Combined effect of TRI or NAC on cytochrome P450-dependent moonooxygenase system in rat liver
Tabela III. Łączny wpływ TRI i NAC na poziom wątrobowego układu monooksygenaz zależnych od cytochromu P450 u szczura

Protein	Time treatment of NAC	Time after exposure [in hours]				
		The control	12	24	48	120
Cytochrome P450	[0 h]	0,83 ± 0,09	0,88 ± 0,02	0,93 ± 0,02	0,89 ± 0,04	0,84 ± 0,06
	[2 h]		0.91 ± 0.02	0.91 ± 0.06	0.96 ± 0.07	0.95 ± 0.07
NADPH-cytochrome P450 reductase	[0 h]	0.09 ± 0.009	0.08 ± 0.005	0.08 ± 0.006	0.08 ± 0.004	0.08 ± 0.005*
	[2 h]		0.08 ± 0.006	0.08 ± 0.007	0.10 ± 0.006	0.10 ± 0.010
Cytochrome b_5	[0 h]	0.76 ± 0.04	0.66 ± 0.03*	0.62 ± 0.06*	0.68 ± 0.04*	0.70 ± 0.04
	[2 h]		0.70 ± 0.07	0.64 ± 0.05*	0.63 ± 0.05*	0.72 ± 0.05
NADH-cytochrome b_5 reductase	[0 h]	0.82 ± 0.03	0.71 ± 0.01*	0.71 ± 0.04*	0.71 ± 0.01*	0.83 ± 0.03
	[2 h]		0.80 ± 0.04	0.81 ± 0.04	0.85 ± 0.05	0.77 ± 0.03

Table IV. Combined effect of TRI, APAP or NAC on cytochrome P450-dependent moonooxygenase system in rat liver
Tabela IV. Łączny wpływ TRI, APAP i NAC na poziom wątrobowego układu monooksygenaz zależnych od cytochromu P450 u szczura

Protein	Time treatment of NAC	Time after exposure [in hours]				
		The control	12	24	48	120
Cytochrome P450	[0 h]	0.83 ± 0.09	0.96 ± 0.06	0.99 ± 0.03	0.94 ± 0.03	0.90 ± 0.04
	[2 h]		1.05 ± 0.04	0.93 ± 0.08	0.95 ± 0.07	0.90 ± 0.07
NADPH-cytochrome P450 reductase	[0 h]	0.09 ± 0.009	0.09 ± 0.006	0.10 ± 0.009	0.11 ± 0.006*	0.10 ± 0.008
	[2 h]		0.09 ± 0.007	0.09 ± 0.007	0.09 ± 0.003	0.10 ± 0.001
Cytochrome b_5	[0 h]	0.76 ± 0.04	0.62 ± 0.04*	0.66 ± 0.04*	0.68 ± 0.05*	0.73 ± 0.08
	[2 h]		0.70 ± 0.06	0.69 ± 0.06	0.74 ± 0.04	0.80 ± 0.07
NADH-cytochrome b_5 reductase	[0 h]	0.82 ± 0.03	0.84 ± 0.06	0.75 ± 0.06	0.70 ± 0.09	0.81 ± 0.05
	[2 h]		0.82 ± 0.06	0.91 ± 0.04*	0.89 ± 0.04*	0.82 ± 0.09

Discussion

In our study, trichloroethylene was not cytochrome P450 stimulant. A long time ago it was found that increased TRI transformation into metabolites secreted with urine, takes place simultaneously with an increase of hepatic cytochrome P450 level [18, 19], however, we did not show an increase of this protein level. Exposure to TRI did not induce significant alterations in cytochrome P450 content, although, there may have been a change in TRI metabolism rate in rat liver, since trichloroethylene has effects on its own metabolism. Literature data suggest that TRI may be CYP inducer, however, what is the ultimate effect of that chloric solvent on cytochrome P450 isoforms is not fully understood [20, 21].

As it is known, trichloroethylene is not only metabolized by monooxygenase cytochrome P450 related system [9, 22] but also increases activity of peroxisomal enzymes. The process is accompanied by intensified, even several times increased catalase activity. Thus, it is likely that qualitative differences in hepatic cancerogenesis related with trichloroethylene, are combined with qualitative differences in peroxisomal proliferation. What is more, increased cell proliferation observed following TRI administration can lead to cell promotion „activated" by reactive oxides and thus increase possibility of neoplastic alterations.

The relation of these observations with possible health risk in people concerned with TRI effect is still partly unclear [23]. Although, peroxisomes proliferation is vital in hepatic carcinoma development, based on some data in literature, TRI is suggested not to pose a great risk for cancerogenesis in humans. It was shown in several epidemiological examinations which did not show increase in hepatic carcinoma incidence in people exposed to TRI [24].

Therefore, we may suggest that the TRI dose we used, seems to have no hepatotoxic effect. The toxicity develops only after administration of a dose which cannot be detoxified [25–27]. Literature data suggest that liver damage caused by TRI is the greatest after 24 hours following exposure to this solvent and recovery takes at least another 24 hours [28, 29]. Stable level of cytochrome P450 and NADPH-cytochrome P450 reductase activity for at least 48 hours since xenobiotic administration, indirectly suggest the lack of degenerative alterations in hepatic lobule.

In a single dose of 250 mg/kg APAP exerted a stimulating effect on cytochrome P450 level, but in a double dose it was highly stimulated [29]. It demonstrates the evidence that rats are relatively resistant to this compound [30, 31]. This high resistance, at least partially, is due to the fact that indirect metabolite mentioned above is effectively conjugated with GSH [32].

Despite elevated level of cytochrome P450 in a group with paracetamol we revealed control levels of NADPH-cytochrome P450 reductase activity. Therefore, we suggest that with the applied APAP dose appears already advanced lipid peroxidation, related with paracetamol-induced liver damage mechanism [33].

N-acetylcysteine was successfully used in clinical examinations in paracetamol overdose (21). We demonstrated that NAC since 12 hour exhibited at least a tendency to decrease cytochrome P450 level, with simultaneous retention of NADPH-cytochrome P450 reductase activity on control level.

If NAC was administered following exposure to TRI, the results were slightly different. Firstly, general cytochrome P450 level tended to increase, though NADPH-cytochrome P450 reductase activity tended to decrease. We also demonstrated cytochrome b_5 levels lower than control and NADH-cytochrome b_5 reductase activity.

In our opinion, lack of alterations of cytochrome P450 level in animal group exposed simultaneously to APAP and TRI is of great significance, whereas at the same time NADPH-cytochrome P450 activity increased. Probably, it is necessary to retain optimal activity of hepatic monooxygenase system, dependent on cytochrome P450 in critical conditions. What is more, a long time ago, Ogino et al. [34] demonstrated that TRI induces lipid peroxidation in the liver and peroxidation itself is catalyzed by NADPH-cytochrome P450 reductase. As a final result, activity of the latter decreases, although with simultaneous exposure to APAP, as we showed, this activity is increased.

In animals treated with a variety of P450 cytochrome related hepatic monooxygenase inducers, it was found that increased TRI transformation to metabolites secreted with urine, progresses simultaneously with an increase of cytochrome P450 hepatic level [35]. Low levels of unbound trichloroethanol (TRI metabolite [36]) secreted by rats undergoing induction, confirm a common observation that cytochrome P450 inducers increase activity of other enzymatic systems, such as UDP-glucuronyltransferase. Increase in the activity of these enzymes causes nearly complete binding of trichloroethanol with glucuronic acid and faster elimination of trichloroethanol in the form of conjugates.

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