

Influence of acetaminophen and trichloroethylene on liver cytochrome P450-dependent monooxygenase system[★]

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The aim of the study was to evaluate the effect of acetaminophen (APAP) and/or trichloroethylene (TRI) on the liver cytochrome P450-dependent monooxygenase system, CYP2E1 and CYP1A2 (two important P450 isoforms), and liver glutathione (GSH) content in rats.

Rats were given three different doses of APAP (250, 500 and 1000 mg/kg b...) and then the above-mentioned parameters were measured for 48 h. The lowest APAP dose produced small changes in the cytochrome P450 content of liver. At 500 mg/kg APAP increased the cytochrome P450 content to 230% of the control. The inductive effect was seen at 1000 mg/kg dose but at 24 h and later. NADPH-cytochrome P450 reductase activity was the highest after the lowest dose of APAP, while after the highest dose it was equal to the control value.

TRI increased both the cytochrome P450 content and the NADPH-cytochrome P450 reductase activity. When TRI was combined with APAP, both these parameters increased in the first hours of observation, but they returned to the control values at 24 h.

When APAP was given at 250 mg/kg, GSH levels decreased to 55% of the control at 8 h and returned to the control values at 24 h. The higher doses of APAP decreased GSH levels more than the lowest dose, but after 24 h GSH levels did not differ from those of the control.

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Abbreviations: APAP, acetaminophen, paracetamol; TRI, trichloroethylene; GSH, glutathione; DNTB, 5,5'-dithiobis-(2-nitrobenzoic acid); SDS, sodium dodecyl sulphate.

When TRI was given at 250 mg/kg, the GSH levels decreased to 68% of the control at 2 h and then they increased gradually and tended to exceed the control values at 48 h.

The effect of TRI combined with APAP on the level of GSH was virtually the same as that of APAP alone given at 500 mg/kg.

Acetaminophen (paracetamol, APAP) is an analgesic and antipyretic drug. It is safe at therapeutic doses, but when overdosed, it damages the liver. In the liver APAP is converted by cytochrome P450 to the toxic compound *N*-acetyl-*p*-benzoquinone imine (Dahlin *et al.*, 1984), which is inactivated by coupling to glutathione (GSH) (Dahlin *et al.*, 1984; Nelson *et al.*, 1990). At higher doses of APAP, the detoxifying capacity of the liver is saturated and APAP metabolites accumulate. APAP hepatotoxicity is therefore closely related to the hepatic level of glutathione (Kuralay *et al.*, 1998).

In the liver, exo- and endogenous compounds are metabolized by the cytochrome P450-dependent monooxygenase system. The enzymes involved in this process are located in the endoplasmic reticulum of the liver, and their activities are dependent on many environmental factors (Plewka & Bienioszek, 1994; Plewka *et al.*, 1994; Plewka & Kamiński, 1996; Plewka *et al.*, 1998).

When considering biotransformation processes, it is important to analyze them together with other cellular processes taking place in the hepatocyte. Impairment of these processes may disturb cellular nutrition (Guengerich, 1995; Plewka & Kamiński, 1996) thus affecting the detoxication reactions (Tamburini & Schenkman, 1986; Geoptar *et al.*, 1995).

It is well known that glutathione plays an important role in maintaining hepatocyte integrity (Comporti *et al.*, 1991; Amimoto *et al.*, 1995). The availability of this tripeptide for generating complexes with reactive species is often a critical factor for the toxicity of many xenobiotics. It has even been suggested that the level of GSH plays a significant role in determining the detoxifying capacity of the organism (Rikans & Kasanka, 1984).

Reduced glutathione acts as a reducing factor in the metabolism of many peroxides. This reduction is catalyzed by glutathione peroxidases (Halliwell & Gutteridge, 1989). A significant decrease in the level of intracellular GSH may lead to oxidative stress. On the other hand, the one-electron reduction of APAP by cytochrome P450 generates reactive oxygen species, thus leading to excess loss of thiol groups and eventual hepatotoxicity (Moore *et al.*, 1985; Corcoran & Wong, 1986).

The aim of this study was to evaluate the effect of APAP and/or TRI (Bruckner *et al.*, 1989) on the activity and composition of the cytochrome P450-dependent monooxygenase system and on the GSH content of liver. We wanted to determine whether these two xenobiotics interact with each other, which would increase their hepatotoxic effects, and whether APAP, whose transformation is mediated by cytochrome P450, influences some P450 isoforms.

MATERIALS AND METHODS

Materials. All chemicals and reagents used were of analytical grade. The rats were obtained from Animal Husbandry, Department of Toxicology, Karol Marcinkowski University of Medical Sciences (Poznań, Poland).

Experimental procedures. The study was performed in the spring season on adult male Wistar rats weighing approximately 270 g \pm 10%. The animals were kept under standard conditions (humidity 60%, temperature 22 \pm 2°C, 12/12 h light/dark cycle) and were fed the standard granulated Murigran chow with access to water *ad libitum*.

Acetaminophen at a dose of 250, 500 or 1000 mg/kg b.m., separately or in combination with trichloroethylene at a dose of 250 mg/kg

b.m. was given intragastrically by stomach tube in a single application.

The rats were killed by decapitation after 2-, 4-, 8-, 12-, 24-, 36 and 48 h after drug(s) application.

Liver microsomal fraction was isolated according to Dallner (Dallner, 1974) and content of cytochrome P450 was determined by the method of Estabrook and Werringloer (Estabrook & Werringloer, 1978). The activity of NADPH-cytochrome P450 reductase was determined by the method of Hodges and Leonard (Hodges & Leonard, 1974). The amount of cytochrome was expressed in nanomoles of cytochrome per milligram of microsomal protein, whereas the activity of the reductase was expressed in micromoles of reduced cytochrome *c* per 1 min for 1 mg of microsomal protein.

The concentration of microsomal protein was measured by the method of Lowry (Lowry *et al.*, 1951) with bovine albumin as a standard.

Glutathione measurement. Total glutathione content (reduced and oxidized) was assayed as described by Adams (Adams *et al.*, 1983). Tissue samples (about 50 mg) were homogenized in 5 ml of 100 mM phosphate buffer, pH 7.5, with 5 mM EDTA (buffer A), which contained 10 mM 5,5'-dithiobis-(2-nitrobenzoic acid) (DNTB). After 10-fold dilution with buffer A, the solution was centrifuged at $2000 \times g$ for 3 min. An aliquot (0.1 ml) was added to cuvette containing 0.1 ml glutathione disulfide reductase (0.5 U/ml) (ICN, 120 U/mg) in buffer A and 0.1 ml 5 mM DNTB in buffer A. The reaction was started with the addition of 0.7 ml 0.3 mM NADPH and the change of absorbance (ΔA) was monitored continuously for 6 min at 412 nm using a U-3210 spectrophotometer (Hitachi). Reference cuvette contained equal concentrations of DNTB, NADPH, enzyme but no sample. Glutathione content was calculated using the calibration curve representing values of $\Delta A/6$ min plotted *versus* known concentrations of

glutathione. The results were expressed in mg/g tissue.

Western blot analysis of P450 isoforms. The levels of liver microsome P450 isoforms were measured by Western blot analysis. Microsome protein samples (5 μ g of protein) were separated on an 8% polyacrylamide gel in the presence of SDS as described by Laemmli (1970) After separation, the proteins were transferred onto a PVDF membrane (Millipore) and immunostained. Rabbit antibodies directed to CYP isoforms were purchased from Chemicon Int. Inc. The bound antibodies were probed with a secondary antibody conjugated with alkaline phosphatase and then visualized with BCIP/NBT (Sigma). The blots were scanned and analyzed densitometrically with a One D Scan software (Scanalytics).

Statistical analysis. The results obtained were evaluated statistically. Differences between groups were assessed by one way analysis of variance (ANOVA) followed by Kruskal-Wallis test to compare all groups to a control or Tukey's test to compare each group to every other group.

The results were expressed as the arithmetic mean value of six independent measurements ($\bar{x} \pm S.D.$, $n = 6$). The statistically significant differences at $P < 0.05$ were marked with a star.

RESULTS

Cytochrome P450-dependent monooxygenase system

The 2 h after acetaminophen administration no changes in cytochrome P450 levels were observed in all examined groups. The lowest dose of this drug elevated slightly contents of this cytochrome at 4 h and 8 h and then they decreased to control values. After 48 h cytochrome P450 level increased again to 115% of control group (Table 1).

Table 1. Effects of different doses of acetaminophen and/or trichloroethylene on rat liver cytochrome P450 content assayed at different times after treatment.

Cytochrome content is expressed in nmol/mg microsomal protein.

	Acetaminophen			TRI	TRI + APAP
	250 mg/kg	500 mg/kg	1000 mg/kg	250 mg/kg	250 mg/kg + 500 mg/kg
Control group	0.715 ± 0.032				
2 h	0.684 ± 0.016	0.698 ± 0.040	0.686 ± 0.041	0.667 ± 0.042	0.665 ± 0.022
4 h	0.914 ± 0.078*	1.068 ± 0.069*	0.665 ± 0.015	0.489 ± 0.078*	0.625 ± 0.045
8 h	0.847 ± 0.040*	1.269 ± 0.214*	0.861 ± 0.049	0.765 ± 0.089	0.845 ± 0.032
12 h	0.673 ± 0.037	1.671 ± 0.107*	0.733 ± 0.013	0.916 ± 0.046*	0.986 ± 0.063*
24 h	0.654 ± 0.017	0.895 ± 0.141*	0.890 ± 0.014*	1.137 ± 0.079*	1.091 ± 0.027*
36 h	0.742 ± 0.024	0.942 ± 0.039*	0.950 ± 0.028*	0.943 ± 0.023*	0.687 ± 0.036
48 h	0.823 ± 0.017*	1.089 ± 0.028*	1.001 ± 0.040*	0.938 ± 0.124*	0.720 ± 0.024

* $P < 0.05$ when compared with control.

At the 2-fold higher dose the cytochrome P450 levels were elevated at all groups (without the 2 h one). The highest APAP dose induced the cytochrome at 24 h and later. The TRI administration decreased the cytochrome P450 content at 4 h only. At 12 h and later we observed its induction. The highest P450 levels were seen at 24 h (160% of control value; Table 1). When given together, TRI and APAP induced the cytochrome at 12 h and 24 h only. Later on, this effect quickly disappeared so that P450 level returned to normal at 36 h.

NADPH-cytochrome P450 reductase activity increased as early as 2 h after APAP given at 250 mg/kg (Table 2). At 48 h the activity was still elevated (no statistically significant difference). When APAP was given at 500 mg/kg, the induction of this reductase activity at all examined groups was observed (without the 4 h one). The highest dose of APAP had no effect on NADPH-cytochrome P450 reductase activity.

TRI increased NADPH-cytochrome P450 reductase activity at 4 h and at later. When

Table 2. Effects of different doses of acetaminophen and/or trichloroethylene on rat liver NADPH-cytochrome P450 activity assayed at different times after treatment.

NADPH-cytochrome P450 activity is expressed in micromol/min per mg microsomal protein.

	Acetaminophen			TRI	TRI + APAP
	250 mg/kg	500 mg/kg	1000 mg/kg	250 mg/kg	250 mg/kg + 500 mg/kg
Control group	0.1009 ± 0.0081				
2 h	0.1216 ± 0.0099*	0.1222 ± 0.0133*	0.1124 ± 0.0101	0.1030 ± 0.0063	0.1192 ± 0.0076*
4 h	0.1375 ± 0.0010*	0.1017 ± 0.0143	0.1137 ± 0.0157	0.1224 ± 0.0024*	0.1249 ± 0.0043*
8 h	0.1505 ± 0.0122*	0.1284 ± 0.0052*	0.1125 ± 0.0151	0.1291 ± 0.0109*	0.1392 ± 0.0040*
12 h	0.1455 ± 0.0050*	0.1385 ± 0.0033*	0.0945 ± 0.0136	0.1285 ± 0.0147*	0.1097 ± 0.0096
24 h	0.1672 ± 0.0483*	0.1216 ± 0.0120	0.1050 ± 0.0086	0.1404 ± 0.0195*	0.1117 ± 0.0043
36 h	0.1478 ± 0.0071*	0.1476 ± 0.0056*	0.1065 ± 0.0052	0.1543 ± 0.0185*	0.1423 ± 0.0137*
48 h	0.1186 ± 0.0139	0.1128 ± 0.0200	0.1072 ± 0.0034	0.1531 ± 0.0134*	0.1364 ± 0.0061*

* $P < 0.05$ when compared with control.

APAP was combined with TRI, the reductase activity increased to 140% until 8 h, and then it returned to the control value, but at 36 h and later it was elevated again.

Glutathione

All examined doses of APAP decreased GSH levels at 8 h, but at 24 h they returned to the control values (Table 3). Effects of the higher doses of APAP were greater than the lowest one.

TRI decreased GSH levels as early as 2 h after treatment (68% of the control value). After

of this isoform decreased to 80% of the control value.

TRI combined with APAP had no effect on CYP1A2 at 24 h, but after 48 h its level decreased to 45% of the control value.

CYP2E1 was not affected by any of the treatments studied.

DISCUSSION

TRI is an organic solvent used in industry and therefore is a common atmospheric pollutant. Metabolic processes play an essential

Table 3. Effects of different doses of acetaminophen and/or trichloroethylene on rat liver glutathione content assayed at different times after treatment.

Glutathione content is expressed in mg/g tissue.

	Acetaminophen			TRI	TRI + APAP
	250 mg/kg	500 mg/kg	1000 mg/kg	250 mg/kg	250 mg/kg + 500 mg/kg
Control group	0.93 ± 0.09				
2 h	0.75±0.06*	0.58±0.09*	0.42±0.08*	0.63±0.07*	0.51±0.04*
8 h	0.50±0.04*	0.29±0.04*	0.25±0.06*	0.76±0.08	0.40±0.08*
24 h	1.05±0.10	1.18±0.20	0.85±0.08	0.82±0.08	1.19±0.18
48 h	1.10±0.09	1.20±0.19	1.01±0.10	1.03±0.12	1.06±0.11

* $P < 0.05$ when compared with control.

wards, GSH level returned to the control value.

The changes caused by TRI combined with APAP were similar to those after APAP alone given at 500 mg/kg.

CYP1A2 and CYP2E1

Since the most significant changes in cytochrome P450 levels were observed in rats treated with APAP at 500 mg/kg the CYP isoforms were studied only in this group of animals.

The level of CYP1A2 showed no changes at 8 h, whereas at 24 h it decreased to 85% and at 48 h to 70% of the control value. TRI had no effect on CYP1A2 at 24 h, but after 48 h the level

role in triggering TRI cytotoxicity. Cytochrome P450 isoforms are key enzymes in the formation of chloral hydrate, the first product of TRI metabolism, which limits the rate of this reaction and consequently the rate of TRI metabolism. Chloral hydrate is also responsible for the hepatotoxicity and carcinogenicity of TRI.

In this study, TRI induced cytochrome P450, which was reflected by increased liver cytochrome levels. Some investigators (Koop *et al.*, 1985) found that TRI induces CYP2E1 but it is unclear how TRI affects other cytochrome P450 isoforms.

In this study, TRI did not affect CYP2E1 levels, but it markedly decreased CYP1A2 levels. Both these effects contradict the noticeable in-

crease in total cytochrome P450 content, which is our original finding. Our finding did not confirm the results of other authors (Wang *et al.*, 1996; Hanioka *et al.*, 1997). This increase is probably a result of induction of CYP2B1/2 (unpublished data) and CYP2C11, another constitutive P450 isoform (Wang *et al.*, 1996). Other authors (Miller & Guengerich, 1983; Guengerich *et al.*, 1991; Nakaji-

critical situation. Ogino (Ogino *et al.*, 1991) and Rouisse & Chakrabarti (Rouisse & Chakrabarti, 1986) have shown that TRI induces lipid peroxidation in the liver and decreases the activity of NADPH-cytochrome P450 reductase (an enzyme that catalyzes lipid peroxidation).

High doses of APAP are hepatotoxic. APAP is converted by cytochrome P450 isoforms to

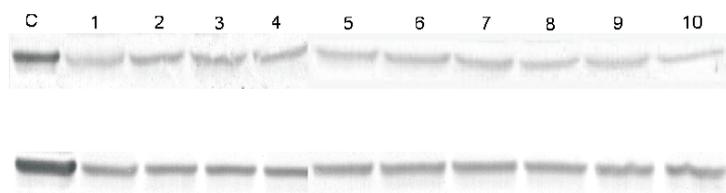


Figure 1. Effects of different doses of acetaminophen and/or trichloroethylene on rat liver CYP1A2 (top) and CYP2E1 (bottom) levels in control group (lane 1), 2 h (lanes 2–4), 24 h (lanes 5–7) and 48 h (lanes 8–10) after treatment APAP (500 mg/kg), TRI (250 mg/kg) and APAP-TRI combination, respectively. Lane C, standard.

ma *et al.*, 1992) have shown that other isoforms than reported previously are not involved in the metabolisms of TRI or they play a minimal role (Wang *et al.*, 1996).

The dose of TRI used in this study seems to produce no hepatotoxic effect. Only doses that cannot be detoxified are hepatotoxic (Rouisse & Chakrabarti, 1986; Dekant *et al.*, 1986). Other authors have shown that TRI-induced hepatotoxicity is maximal 22–24 h after exposure, and the pre-exposure state is restored only after 40–45 h (Okino *et al.*, 1991; Elcombe *et al.*, 1985). In this study, cytochrome P450 levels and NADPH-cytochrome P450 reductase activity remained elevated for 48 h since the moment of TRI administration, which indirectly indicates a lack of degenerative changes in the liver.

The animals treated with APAP and TRI combined showed a significant increase in cytochrome P450 levels, at 12 h and at 24 h after treatment (140% of the control; Table 1), while NADPH-cytochrome P450 reductase activity in this groups decreased to the control value. This prolonged increase in cytochrome P450 levels seems to be an attempt to maintain the optimal activity of the cytochrome P450-dependent monooxygenase system in a

N-acetyl-*p*-benzoquinone imine, a toxic compound that damages the liver unless it is bound by GSH (Nelson *et al.*, 1990; Ryu *et al.*, 1998). When given at a single dose of 250 mg/kg, APAP only slightly altered cytochrome P450 content (Table 1), while at 500 mg/kg it strongly induced cytochrome P450. This shows that rats are relatively resistant to APAP (Amimoto *et al.*, 1995). Such a high resistance (also seen at 1000 mg/kg) results at least partially from the fact that *N*-acetyl-*p*-benzoquinone imine is effectively neutralized by GSH, which may be supported by the considerable decrease in GSH levels observed at the same time (Table 3). Thus it seems that GSH is an important factor determining APAP hepatotoxicity and that GSH plays a key role in the detoxification of APAP (Kuralay *et al.*, 1998).

Although cytochrome P450 levels were high in the rats treated with the highest APAP dose, NADPH-cytochrome P450 activity remained unchanged despite the fact that it was induced by lower APAP doses. One can suggest that high doses of APAP result in excessive lipid peroxidation (Amimoto *et al.*, 1995) associated with APAP-induced liver damage. This effect is independent of the hepatotoxic

effect of APAP metabolites and therefore in this cases the protective action of GSH is ineffective.

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