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Metabolic Interactions between Acetylsalicylic Acid, Xylene and Trichloroethylene in Rats

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Abstract

This study aimed at evaluating the effect of acetylsalicylic acid (ASA) (150 and 300 mg/kg b.w.) on urinary trichloroethanol and trichloroacetic acid excretion and the liver cytochrome P-450-dependent monooxygenase system (MFO) in rats treated with trichloroethylene (TRI) alone or with xylene (XYL) at a concentration of 4.5 mmol/m^3 air. The study has shown that xylene equally decreased trichloroacetic acid and trichloroethanol excretion within 48 hours after exposure. Acetylsalicylic acid diminished the excretion of both trichloroethylene metabolites in a dose-dependent manner, although the effect was weaker than that of xylene.

Liver cytochrome P-450 content tended to increase after both doses of ASA. There were no significant changes in cytochrome b_5 content and the activities of NADPH-cytochrome P-450 and NADH-cytochrome b_5 reductases. TRI decreased cytochrome P-450 and cytochrome b_5 contents and reduced both reductase activities. XYL induced all MFO components.

Acetylsalicylic acid at 150 mg/kg combined with TRI inhalation tended to lower cytochrome b_5 content and NADH-cytochrome b_5 reductase activity. When given at 300 mg/kg, ASA increased cytochrome P-450 content, while cytochrome b_5 content and NADH-cytochrome b_5 reductase activity were still decreased, but to a smaller degree when compared with the lower ASA dose.

XYL together with the lower dose of ASA induced the MFO system. Exposure to XYL and the higher dose of ASA elevated cytochrome P-450 content and NADPH-cytochrome P-450 reductase activity and it diminished NADH-cytochrome b_5 reductase activity.

In rats treated simultaneously with ASA, XYL and TRI both cytochromes increased in amount, while the other components of the MFO system did not change.

Keywords: acetylsalicylic acid, trichloroethylene, xylene, trichloroethanol, trichloroacetic acid, cytochrome P-450-dependent monooxygenases

Introduction

Acetylsalicylic acid (aspirin, ASA), a drug with analgetic, antipyretic, antiinflammatory, and antiaggregatory properties, has been used for many years in the treatment of many diseases, including cerebrovascular and coronary artery diseases. Organic solvents such as trichloroethylene, toluene and xylene, are also widely used in industry and household. As a result, there is a possibility for humans to be exposed to a combination of acetylsalicylic acid and organic solvents, resulting in unexpected interactions and effects occurring at various stages of toxic processes [1-6].

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In our previous studies we focused on mutual interactions between organic solvents and their effects on metabolic processes, including excretion of major metabolites used as biomarkers of exposure [4-13].

The aim of this study was to evaluate the influence of acetylsalicylic acid and xylene on the urinary excretion of trichloroacetic acid and trichloroethanol and on the liver cytochrome P-450-dependent monooxygenase system in rats exposed to trichloroethylene vapors.

Materials and Methods

Materials

All chemicals and reagents were of analitycal grade: trichloroethylene (Ubichem), trichloroacetic acid (Carl Roth KG), trichloroethanol (Aldrich Chemie), acetylsalicylic acid (Merck), carbon disulphide (Merck), chromium trioxide (Sigma), pyridine (Loba Feinchemie), and p-toluidine hydrochloride (Aldrich Chemie).

Rats were obtained from Animal Husbandry, Department of Toxicology, Karol Marcinkowski University of Medical Sciences in Poznan, Poland.

Experimental Procedures

The study was performed on adult male Wistar rats weighing approximately 270 g (± 10%). The animals were housed in standard conditions (60% humidity, 22 ± 2°C, 12/12 hours light/dark cycle) and fed the standard granulated chow Murigran and water *ad libitum.*

The animals were exposed to solvent vapours in a dynamic toxicological chamber (a capacity of 126 dm^3) at 21°C and with 15 air changes per hour. The solvent concentrations inside the chamber were measured every 30 minutes by gas chromatography [10, 11].

The animals inhaled according to the following regimen:

- trichloroethylene at 4.5 mmol/m^3 air (591.3 mg/m^3 = 12 TLV - treshold limit value)
- trichloroethylene at 4.5 mmol/m^3 air following acetyl salicylic acid at 150 mg/kg b.w. (1/10 LD_{50})
- trichloroethylene at 4.5 mmol/m^3 air following acetyl salicylic acid at 300 mg/kg b.w. (1/5 LD_{50})
- trichloroethylene combined with xylene, both at 4.5 mmol/m^3 air (591.3 mg/m^3 = 12 TLV and 487 mg/m^3 = 4.8 TLV, respectively)
- trichloroethylene combined with xylene, both at 4.5 mmol/m^3 air following acetylsalicylic acid at 150 mg/kg b.w.
- trichloroethylene combined with xylene, both at 4.5 mmol/m^3 air following acetylsalicylic acid at 300 mg/kg b.w.

Before inhalation, rats were given 3 cm^3 of aqueous suspension of acetylsalicylic acid by a stomach tube. The control rats were given only distilled water. Rats inhaled for 5 days, 6 hours a day (8^{00} - 14^{00}). Urine samples were collected 24 and 48 hours after cessation of exposure for measurement of trichloroacetic acid and trichloroethanol levels [7].

Sixty-six hours after inhalation, the rats were killed by decapitation, always between 8:30 and 9:30 a.m. to avoid circadian fluctuations in the activity of mixed-func-tion-oxidase (MFO) system [14]. Liver microsomal fractions were isolated as described by Dallner [15] and cytochrome P-450 and b_5 levels were determined by the method of Estabrook and Werringloer [16]. The activities of NADPH-cytochrome P-450 reductase and NADH-cytochrome b_5 reductase were determined as described by Hodges and Leonard [17]. The cytochrome levels were expressed in nanomoles per 1 mg of microsomal protein, whereas the reductase activities were expressed in micromoles of reduced cytochrome c per 1 min/mg of microsomal protein. Microsomal protein content was measured by the method of Lowry *et al.* [18] with bovine albumin as a standard.

Statistical analysis was performed with the Kolmogorow test to confirm the normal distribution of the results and the Tuckey multiple comparison test to compare the means [19]. Student's t-test was used to compare the results of components of the monooxygenases system in different groups.

The results are presented as the arithmetic mean of six independent measurements (x ± SD, n=6). Significant differences ($p<0.05$) are marked with an asterisk (*). Increased and decreased levels of metabolites in urine are marked with arrow symbols, (↑↓), respectively.

Results and Discussion

Urinary trichloroacetic acid (TCA) and trichloroethanol (TCE) excretion in rats exposed to TRI alone or combined with acetylsalicylic acid (ASA) and xylene (XYL) are presented in Tables 1-3, where the results of statistical analysis are also shown.

Compared to the rats exposed to TRI alone, TCA and TCE levels in the rats exposed to TRI and XYL were significantly lower (Table 1). Xylene decreased TCA and TCE levels by about 28% within the first 24 hours and by about 61% (TCA) and 67% (TCE) within the next 24 hours. However, the two metabolites showed similar decreases of total 48-hour excretion (by about 34%). ASA at 150 and 300 mg/kg b.w. decreased TCA and TCE excretion in a dose-dependent manner, though the decrease was smaller than that after XYL. Combined exposure to TRI and XYL following ASA given at a dose of 150 or 300 mg/kg b.w. significantly reduced TCA excretion within the first 24 hours (by about 62%). In the next 24 hours TCA excretion decreased, depending on the dose of ASA (by about 51% at 150 mg/kg b.w. and by about 40% at 300 mg/kg b.w.). After combined exposure to ASA (300 mg/kg b.w.), XYL and TRI, TCE excretion increased by about 13% in the first 24 hours and decreased in the next 24 hours.

Table 2 presents TCA and TCE levels in urine from rats exposed to a combination of TRI, XYL and ASA compared with rats exposed to TRI and XYL. The administration of ASA increased TCE excretion 48 hours after exposure in a dose-dependent manner (by about 43% and 60%, respectively). At the same time, TCA excretion decreased by about 40% independent of the ASA dose.

Table 1. Trichloroacetic acid (TCA) and trichloroethanol (TCE) levels in urine from untreated or acetylsalicylic acid (ASA)-treated rats (150 and 300 mg/kg b.w.) exposed to trichloroethylene (TRI) and xylene (XYL) vapours (both at 4.5 mmol/m^3) in comparison with rats exposed to trichloroethylene (TRI) alone (x ± SD; n = 6).

Exposure	Urinary metabolite levels [mg]					
	TCA			TCE		
	Time of urine collection [h]					
	0 – 24	25 – 48	0 – 48	0 – 24	25 – 48	0 – 48
TRI 4.5 mmol/m^3 x ± SD	8.20 ± 0.33	1.40 ± 0.09	9.60 ± 0.38	16.48 ± 1.07	3.08 ± 0.29	19.55 ± 1.01
TRI 4.5 mmol/m^3 XYL 4.5 mmol/m^3 x ± SD	5.88* ± 0.37	0.55* ± 0.05	6.43* ± 0.41	11.81* ± 0.83	1.02* ± 0.25	12.83* ± 0.80
	Changes in excretion [%]					
	↓ 28.3*	↓ 60.7*	↓ 33.0*	↓ 28.3*	↓ 66.9*	↓ 34.4*
TRI 4.5 mmol/m^3 ASA 150 mg/kg m.c. x ± SD	7.75 ± 0.38	108* ± 0.13	8.83* ± 0.38	14.98* ± 0.62	2.51 ± 0.33	17.49* ± 0.45
	Changes in excretion [%]					
	↓ 5.5	↓ 22.9*	↓ 8.0*	↓ 9.1*	↓ 18.5	↓ 10.5*
TRI 4.5 mmol/m^3 ASA 300 mg/kg m.c. x ± SD	6.37* ± 0.32	0.91* ± 0.06	7.28* ± 0.31	13.26* ± 0.37	1.88* ± 0.29	15.14* ± 0.32
	Changes in excretion [%]					
	↓ 22.3	↓ 35.0*	↓ 24.2*	↓ 19.5*	↓ 38.9*	↓ 22.6*
TRI 4.5 mmol/m^3 XYL 4.5 mmol/m^3 ASA 150 mg/kg m.c. x ± SD	2.99* ± 0.25	0.68* ± 0.07	3.67* ± 0.26	16.84 ± 0.87	1.45* ± 0.25	18.29 ± 0.98
	Changes in excretion [%]					
	↓ 63.5*	↓ 51.4*	↓ 61.8*	↑ 2.2	↓ 52.9*	↓ 6.4
TRI 4.5 mmol/m^3 XYL 4.5 mmol/m^3 ASA 300 mg/kg m.c. x ± SD	3.13* ± 0.09	0.85* ± 0.04	3.97* ± 0.11	18.69* ± 0.97	1.86* ± 0.15	20.4 ± 0.94
	Changes in excretion [%]					
	↓ 61.8*	↓ 39.3*	↓ 58.6*	↑ 13.4*	↓ 39.6*	↑ 5.1

Table 3 presents TCA and TCE levels in urine from rats exposed to a combination of TRI, XYL and ASA compared with the rats exposed to TRI following ASA ingestion. An increase in the ASA dose inhibited the stimulatory effect of XYL on TCA excretion at 48 h (from 58.4% to 46.5%). In contrast, the higher ASA dose increased TCE excretion from 4.6% to 35.7% .

Table 4 shows cytochrome P-450 and cytochrome b_5 levels and activitites of NADPH-cytochrome P-450 and NADH-cytochrome b_5 reductases in the liver microsomal fraction. ASA increased cytochrome P-450 levels in a dose-dependent manner, but it had no effect on cytochrome b_5 and both reductases. TRI decreased the levels of both cytochromes and both reductases.

After ASA (150 mg/kg b.w.) and TRI, there was no change in cytochrome P-450 content, cytochrome b_5 content and NADH-cytochrome b_5 reductase activity decreased, and NADPH-cytochrome P-450 reductase activity increased significantly.

The higher ASA dose (300 mg/kg b.w.) combined with TRI increased the cytochrome P-450 content and decreased the cytochrome *bs* content and NADH-cytochrome b_5 reductase activity. These changes, however, were smaller than those caused by the lower ASA dose.

The rats exposed to XYL showed induction of all components of the MFO system. ASA inhibited the inductory effect of XYL. Combined exposure to XYL and TRI increased cytochrome P-450 and cytochrome b_5 contents and NADPH-cytochrome P-450 reductase activity, but the values were lower than those observed in the rats treated with XYL alone. Combined exposure to both doses of ASA, XYL and TRI induced cytochrome P-450

Table 2. Trichloroacetic acid (TCA) and trichloroethanol (TCE) in urine from untreated or acetylsalicylic acid (ASA)-treated rats (150 and 300 mg/kg b.w.) exposed to trichloroethylene (TRI) and xylene (XYL) vapours (both at 4.5 mmol/m^3) vs. rats exposed to trichloroethylene combined with xylene, (x ± SD; n = 6).

Exposure	Urinary metabolite levels [mg]					
	TCA			TCE		
	Time of urine collection [h]					
	0 – 24	25 – 48	0 – 48	0 – 24	25 – 48	0 – 48
TRI 4.5 mmol/m^3 XYL 4.5 mmol/m^3 x ± SD	5.88 ± 0.37	0.55 ± 0.05	6.43 ± 0.41	11.81 ± 0.83	1.02 ± 0.25	12.83 ± 0.80
TRI 4.5 mmol/m^3 XYL 4.5 mmol/m^3 ASA 150 mg/kg m.c. x ± SD	2.99* ± 0.25	0.68 ± 0.07	3.67* ± 0.26	16.84* ± 0.87	1.45 ± 0.25	18.29* ± 0.98
	Changes in excretion [%]					
	↓ 49.2*	↑ 23.6	↓ 42.9*	↑ 42.6*	↑ 42.2	↑ 42.6*
TRI 4.5 mmol/m^3 XYL 4.5 mmol/m^3 ASA 300 mg/kg m.c. x ± SD	3.13* ± 0.09	0.85* ± 0.04	3.97* ± 0.11	18.69* ± 0.97	1.86* ± 0.15	20.54* ± 0.94
	Changes in excretion [%]					
	↓ 46.8*	↑ 54.5*	↓ 38.3*	↑ 58.3*	↑ 82.4*	↑ 60.1*

Table 3. Trichloroacetic acid (TCA) and trichloroethanol (TCE) levels in urine from rats exposed to trichloroethylene (TRI) and xylene (XYL) vapours (both at 4.5 mmol/m^3 air) following acetylsalicylic acid (ASA) treatment (150 or 300 mg/kg b.w.) vs. TCA and TCE levels in urine from ASA-pretreated rats exposed to TRI alone (x ± SD; n = 6).

Exposure	Urinary metabolite levels [mg]					
	TCA			TCE		
	Time of urine collection [h]					
	0 – 24	25 – 48	0 – 48	0 – 24	25 – 48	0 – 48
TRI 4.5 mmol/m^3 ASA 150 mg/kg m.c. x ± SD	7.75 ± 0.38	1.08 ± 0.13	8.83 ± 0.38	14.98 ± 0.62	2.51 ± 0.33	17.49 ± 0.45
TRI 4.5 mmol/m^3 XYL 4.5 mmol/m^3 ASA 150 mg/kg m.c. x ± SD	2.99* ± 0.25	0.68* ± 0.07	3.67* ± 0.26	16.84* ± 0.87	1.45* ± 0.25	18.29 ± 0.98
	Changes in excretion [%]					
	↓ 61.4*	± 37.0*	↓ 58.4*	↑ 12.4*	↓ 42.2*	↑ 4.6
TRI 4.5 mmol/m^3 ASA 300 mg/kg m.c. x ± SD	6.37 ± 0.32	0.91 ± 0.06	7.28 ± 0.31	13.26 ± 0.37	1.88 ± 0.29	15.14 ± 0.32
TRI 4.5 mmol/m^3 XYL 4.5 mmol/m^3 ASA 300 mg/kg m.c. x ± SD	3.13* ± 0.09	0.85 ± 0.04	3.97* ± 0.11	18.69* ± 0.97	1.86 ± 0.15	20.54* ± 0.94
	Changes in excretion [%]					
	↓ 50.9*	↓ 6.6	↓ 46.5*	↑ 40.9*	↓ 1.1	↑ 35.7*

Table 4. Liver cytochrome P-450-dependent monooxygenase system in rats treated with acetylsalicylic acid (ASA) alone (150 or 300 mg/kg b.w.per *os)* or together with trichloroethylene (TRI) and/or xylene (XYL) vapours (both at 4.5 mmol/m^3 air, 6h/day, 5 days, x ± SD, n=6). Abbreviations: P-450 (cytochrome P-450), NADPH (NADPH-cytochrome P-450 reductase), cyt. b_5 (cytochrome b_5), NADH (NADH-cytochrome b_5 reductase).

Exposure	Cytochrome P-450 µM/mg [P-450]	NADPH-cyt. P-450 reductase µM/min/mg [NADPH]	Cytochrome b_5 µM/mg [cyt. b_5]	NADH-cyt. b_5 reductase µM/min/mg [NADH]
Control group	0.7487 ± 0.0330	0.1052 ± 0.0048	0.6097 ± 0.0162	0.5776 ± 0.0180
ASA 150 mg/kg	0.8108 ± 0.0280*	0.0963 ± 0.0054	0.6647 ± 0.0126	0.5237 ± 0.0174
ASA 300 mg/kg	0.9208 ± 0.0125*	0.0928 ± 0.0018	0.6230 ± 0.0123	0.5030 ± 0.0122
TRI 4.5 mmol/m^3	0.5478 ± 0.0116*	0.0780 ± 0.0069*	0.4230 ± 0.0107*	0.3577 ± 0.0147*
ASA 150 mg/kg TRI 4.5 mmol/m^3	0.6936 ± 0.0129	0.1207 ± 0.0082*	0.1073 ± 0.0082*	0.4066 ± 0.0210*
ASA 300 mg/kg TRI 4.5 mmol/m^3	0.8029 ± 0.0169*	0.1148 ± 0.0086	0.4536 ± 0.0167*	0.4355 ± 0.0156*
XYL 4.5 mmol/m^3	1.0349 ± 0.0789*	0.1825 ± 0.0147*	1.0446 ± 0.0245*	0.7396 ± 0.0039*
ASA 150 mg/kg XYL 4.5 mmol/m^3	1.0584 ± 0.0631*	0.1170 ± 0.0099	0.9459 ± 0.0413*	0.6973 ± 0.0251*
ASA 300 mg/kg XYL 4.5 mmol/m^3	0.9742 ± 0.0457*	0.1280 ± 0.0060*	0.6984 ± 0.0456	0.4789 ± 0.0351*
TRI 4.5 mmol/m^3 XYL 4.5 mmol/m^3	0.9963 ± 0.0620*	0.1234 ± 0.0081*	0.8279 ± 0.0619*	0.5870 ± 0.0225
ASA 150 mg/kg TRI 4.5 mmol/m^3 XYL 4.5 mmol/m^3	0.9282 ± 0.0203*	0.1141 ± 0.0106	0.7118 ± 0.0369*	0.5634 ± 0.0256
ASA 300 mg/kg TRI 4.5 mmol/m^3 XYL 4.5 mmol/m^3	0.9589 ± 0.0328*	0.1189 ± 0.0060	0.8077 ± 0.0860*	0.5950 ± 0.0184

* significantly different from control group, $p < 0.05$.

and cytochrome b_5 and had no significant effect on the reductases.

The effects of acetylsalicylic acid and xylene on trichloroethylene metabolism in rats is not fully understood. Xylene is quickly converted by the liver cytochrome P-450 system to methylbenzyl alcohol that is conjugated with glycine and excreted as methylhippuric acid. CYP2E1 is the main isoform responsible for the formation of methylbenzyl alcohol. Other isoforms including CYP2B1/2 and CYP2C11 take part in this process as well. The same isoforms take part in the metabolism of many xenobiotics, for example toluene induces CYP2E1 and CYP2B1/2 but inhibits CYP2C11; trichloroethylene induces CYP2E1 and CYP2B1/2 (the latter shows weak response) and inhibits other P-450 isoforms [20]. The induction of cytochromes P-450 by phenobarbital and ethanol accelerates the metabolism of trichloroethylene and intensifies its hepatotoxicity [21-22].

In our previous studies [23] toluene did not induce cytochrome P-450, which may be explained by the inhibition of CYP2C11 and simultaneous induction of CYP2E1 and CYPB1/2. We have recently demonstrated that trichloroethylene (1.5 mmol/m^3) decreased liver cytochrome P-450 and cytochrome b_5 contents and NADPH-cytochrome b_5 reductase activity.

Although acetylsalicylic acid is subject to hydrolysis by non-specific esterases [24-26], it significantly increased the cytochrome P-450 content (at 300 mg/kg b.w.) and did not affect the other components of the system.

The drug produced an inductive effect on the cytochrome P-450 content and reductase activities that were decreased by trichloroethylene, and it simultaneously decreased the cytochrome b_5 content in rats treated with ASA and TRI. Combined exposure to trichloroethylene and toluene increased the cytochrome P-450 content and both reductase activities compared with those treated with trichloroethylene alone. ASA increased still further the cytochrome b_5 content. In the case of combined exposure to acetylsalicylic acid, trichloroethylene and xylene, changes were similar to those presented above.

Acetylsalicylic acid is hydrolyzed to salicylic acid that is excreted after conjugation with glycine or glucuronic acid. Salicylic acid may also be subject to hydroxylation to form gentisic acid that is coupled with glycine and ex-

creted as gentysuric acid [25]. The metabolism of acetylsalicylic acid as well as the biotransformation of solvents may be influenced by other xenobiotics [3, 27].

Campbel *et al.* [3], who investigated the influence of acetylsalicylic acid (1500 mg) on methylhippuric acid excretion in humans exposed to xylene100 ppm/4h), found that the excretion of methylhippuric acid-and the drug decreased by about 50%. They linked those changes to phase II biotransformation (conjugation with glycine). It is easier to explain the influence of acetylsalicylic acid on the excretion of toluene and xylene metabolites [4,5]. However, considering the results of this study, we cannot exclude the involvement of ASA in phase II biotransformation either. It is more difficult to explain the influence of acetylsalicylic acid on trichloroethylene biotransformation because the mechanism by which ASA inhibits the conversion of TRI to TCA and TCE is not fully understood. The decrease in TCE excretion together with increasing doses of ASA, may be associated with the intensification of TRI oxidation caused by cytochrome P-450, which result in the formation of chloral hydrate that undergoes further transformations, namely oxidation to TCA or reduction to TCE [22, 28-32].

The ASA-induced increase in the cytochrome P-450 content may, to a greater extent, influence the conversion of TRI to TCA than the conversion of TRI to TCE. However, in our study the excretion of TCA and TCE was reduced. ASA is a weak inducer of cytochrome P-450 [33, 34], an enzyme system that plays an important role in biotransformation of TRI and XYL. To explain this unexpected response to ASA, one should investigate cytochrome P-450 isoforms that are directly involved in the biotransformation of TRI and XYL. The decrease in TCA and TCE excretion may also indicate increased exhalation of unchanged TRI. Moreover, we should bear in mind the fact that ASA may induce not only cytochrome P-450 but also other enzymes, e.g. UDP-glucuronyl--transferase, an enzyme that conjugates TCA and TCE with glucuronic. As a result, both TRI metabolites are excreted as glucuronides. Furthermore, ASA may induce GSH-transferase, which couples TRI with glutathione to form S-(l,2-dichlorovinyl)GSH, which is converted to dichlorovinylcysteine (DCVC) [30].

In light of the results of this study, it is likely that the changes in the excretion of trichloroethylene metabolites caused by combined exposure to trichloethylene, xylene and acetylsalicylic acid result from mutual interactions between these xenobiotics occurring in both phases of biotransformation.

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