Enzymes induced by ethanol differently affect the pharmacokinetics of trichloroethylene and 1,1,1-trichloroethane

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Abstract

This study was undertaken to clarify the effect of enzymes induced by ethanol consumption on the pharmacokinetics of trichloroethylene (TRI, a highly metabolised substance) and 1,1,1-trichloroethane (1,1,1-TRI, a poorly metabolised substance). Rats maintained on a control liquid diet or a liquid diet containing ethanol (2 g/day/rat) for not less than three weeks were exposed to either TRI (50, 100, 500, and 1000 ppm) or 1,1,1-TRI (50, 100, and 500 ppm) by inhalation for six hours and the concentration of each compound in the blood and the urinary excretion of metabolites (trichloroethanol and trichloroacetic acid) were measured over several hours. Ethanol, which increased the *in vitro* metabolism of both compounds about fivefold, enhanced the *in vivo* metabolism of TRI only at high levels of exposure (marginally at 500 and considerably at 1000 ppm), whereas the metabolism of 1,1,1-TRI was enhanced at all concentrations tested. Moreover, there was a definite difference in the effect of induction of enzymes between the two solvents: the enhanced metabolism of TRI *in vivo* was shown by a decrease in the blood concentration of TRI as well as by an increase in the urinary excretion of its metabolites, whereas that of 1,1,1-TRI was shown by an increase in the urinary excretion of its metabolites alone. These results suggest that the induction of enzymes differentially affects the pharmacokinetics of TRI and 1,1,1-TRI in human occupational exposure: TRI metabolism may be increased only at concentrations much higher than the current occupational exposure limit (mostly 50 ppm), whereas 1,1,1-TRI metabolism may be increased at an exposure similar to occupational exposure.

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Hepatic metabolism of organic solvents plays a decisive part in determining the disappearance rate of most organic solvents from the body.¹ Enzymes that regulate the biotransformation are mono-oxygenases related to cytochrome P-450 and are most concentrated in the liver.² The metabolic pathway whereby the cytochrome P-450 system is involved is considered to be the rate limiting step in the

overall metabolic processes, because it requires energy to cleave chemical bonds. This enzyme system is susceptible to the influence of various environmental factors including dietary and drinking habits, medication, and exposure to environmental chemicals.³

Ethanol consumption is probably one of the most important environmental factors that affect the pharmacokinetic behaviour of organic solvents in humans as ethanol is the only biologically active substance that large numbers of industrial workers often consume in multigram quantities. Some workers may have consumed large amounts of alcoholic beverages in the evening and are then exposed to organic solvent vapours in the workplace the next day.

Ethanol consumption induces a form of cytochrome P-450 (P-450IIE1),⁴ which is one of the constitutive microsomal enzymes in both humans⁵ and other animals.⁶ It is characterised by its high affinity (low-K_m enzyme) for low molecular weight substances, including organic solvents.⁷

Experimental evidence has shown that ethanol given to rats accelerates the hepatic metabolism *in vitro* of a variety of volatile organic solvents including trichloroethylene (TRI) and 1,1,1-trichloroethane (1,1,1-TRI).⁸ The metabolism enhancing effect of ethanol has also been shown in *in vivo* metabolism studies.^{9,10} For example, TRI disappeared faster in ethanol treated, than in control rats when they were exposed to air containing TRI at 500 ppm or higher, and the ethanol treated rats excreted much more trichloroacetic acid (TCA) and trichloroethanol (TCE), the major metabolites of TRI, than did the control rats.¹⁰ These results should be interpreted with caution, however, as occupational exposure to concentrations as high as 500 ppm is unusual.

A simulation study with a physiologically based pharmacokinetic model has shown that the effect of induction of enzymes on the metabolism of inhaled chemicals depends on the chemical as well as on the level of exposure.^{11,12}

The rate of 1,1,1-TRI metabolism is about 1/40 that of TRI metabolism.⁸ TRI is categorised as a highly metabolised substrate whereas 1,1,1-TRI is poorly metabolised.¹² In our study, we further investigated the working hypothesis that enzyme induction by ethanol consumption may affect the pharmacokinetics of 1,1,1-TRI differently from that of TRI.

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Materials and methods

ANIMALS AND DIETS

Eight week old male Wistar rats (Shizuoka Laboratory Animal Centre, Shizuoka) were kept individually in stainless steel wire bottomed metabolism cages in an air conditioned room (22(2)°C) with artificial lighting from 0600 to 1800. They were maintained on unlimited pellet food (Clea CE-2, Nippon Clea, Tokyo) and water. At 10 weeks of age, they were switched to two different liquid diets; one group of rats to a well balanced liquid diet (control diet) and the other group to a diet containing ethanol (ethanol diet). The control diet was prepared by modifying that described by Lieber and DeCarli.¹³ Dextrin and maltose were replaced with sucrose and ethyl linoleate was substituted for safflower oil. The control diet (1 kcal/ml, 80 ml), which constituted food and water for one day, contained 3.31 g casein, 3.17 g oil (corn oil, olive oil, and ethyl linoleate), 9.22 g sucrose, 40 mg L-cystine, 24 mg DL-methionine, 42 mg choline bitartrate, 0.8 g fibre (KC-flock, Sanyo Kokusaku Pulp, Tokyo), 240 mg xanthan gum, 7.2 mg vitamin mixture, and 570 mg mineral mixture. The ethanol diet (1 kcal/ml) was identical to the control diet except that it contained 2.0 g ethanol with a reduction of sucrose content to 3.07 g and an increase of oil content to 4.34 g/80 ml. Thus the ethanol diet was an ethanol containing, low carbohydrate, high fat diet, a prescription that is effective in inducing the ethanol inducible cytochrome P-450.¹⁴

After three weeks on the liquid diets, rats were either killed for a metabolism study in vitro or exposed to TRI or 1,1,1-TRI by inhalation for kinetic studies in vivo. On the day of the kinetic studies, all the rats exposed to TRI or 1,1,1-TRI were maintained on the control diet. The experiments were performed in accordance with the guidelines for animal experiments, Medical University of Yamanashi.

METABOLISM OF TRI AND 1,1,1-TRI IN VITRO

Rats were killed by decapitation at 1000 and the liver was removed. A 25% (w/v) liver homogenate in 1.15% KCl - 0.01 M phosphate buffer (pH 7.4) was centrifuged at 10 000 *g* for 10 minutes. The supernatant was further centrifuged at 105 000 *g* for 60 minutes to obtain a microsomal pellet that was suspended in the buffer and centrifuged again at 105 000 *g* for 60 minutes. The washed microsomal pellet was resuspended in the same buffer and the protein content was measured by the method of Lowry *et al.*¹⁵ The protein content was adjusted to a concentration of 10 mg/ml with the buffer, aerated with N₂, and stored frozen at -85°C until use.

Microsomal cytochrome P-450 content was determined spectrophotometrically by the method of Omura and Sato.¹⁶ TRI and 1,1,1-TRI metabolism in vitro was assessed by measuring the rate of substrate (TRI or 1,1,1-TRI) disappearance by the method of Sato and Nakajima¹⁷ with the slight modification that the final volume of reaction mixture

was reduced to 0.5 ml consisting of 0.1 ml of microsomal solution (enzyme), 0.3 ml of cofactor solution, and 0.1 ml of substrate solution. The reaction mixture (0.5 ml) contained a final concentration of 1.0 mM NADP, 50 mM MgCl₂, 20 mM glucose 6-phosphate (G-6-P), 50 mM K/K phosphate buffer, and 0.11 mM TRI or 0.10 mM 1,1,1-TRI in addition to two units of G-6-P dehydrogenase and microsomes corresponding to 1.0 mg protein.

EXPOSURE TO TRI AND 1,1,1-TRI BY INHALATION

Both control and ethanol treated rats were exposed to air containing TRI at a concentration of 50, 100, 500, or 1000 ppm, or 1,1,1-TRI at a concentration of 50, 100, or 500 ppm for six hours from 1000 to 1600. The exposure was in a dynamic flow exposure chamber as described previously.¹⁸ At pre-selected intervals after the end of exposure, a small amount of blood (20 µl) was taken from a cut in the tail, and TRI or 1,1,1-TRI concentration in the blood was measured by means of a gas chromatographic syringe equilibration method.¹⁹ The operating conditions of the gas chromatograph were: 2 m × 3 mm glass column packed with PEG-400 on Unipor B (Gasukuro Kogyo, Tokyo) at 80°C; injection port temperature, 100°C; carrier gas, N₂ at 70 ml/min; H₂ at 20 ml/min.

Samples of urine collected at predetermined intervals from the start of exposure for 48 hours were each diluted with distilled water to 100 ml, then centrifuged at 3000 rpm for five minutes. The major urinary metabolites of both TRI and 1,1,1-TRI (TCA and TCE) were analysed in the urine essentially according to the head space gas chromatographic method originally described by Breimer *et al.*²⁰ as modified by Ohara *et al.*²¹ In brief, 0.1 ml of diluted urine was placed in a head space vial (Perkin-Elmer; 22 ml in volume) containing 0.05 ml methanol and 0.25 ml concentrated H₂SO₄. The vial, capped with a Teflon lined stopper, was kept at 85°C to make the methyl ester of TCA and to hydrolyse TCE-glucuronide. The head space gas was transferred to a gas chromatograph (Hitachi 263-30) equipped with an electron capture detector (⁶³N), and analysed for TCA and TCE. This series of procedures was performed with an autosampler (Perkin-Elmer Headspace Sampler HS 40). The resultant peak areas were measured with an integrator (Hitachi Chromato-Integrator D-2500). Operating conditions of the autosampler were: thermostat temperature 85°C; thermostat time 65 minutes; transfer temperature 120°C; pressurisation time 0.5 minutes; injection time 4.8 seconds. The analytical conditions of the gas chromatograph were: column 0.32 mm × 30 m TC-WAX capillary (GL Sciences, Tokyo); column temperature 130°C; carrier gas, N₂ at a flow rate of 2 ml/min; split ratio, 40:1.

STATISTICS

The results are represented by means (SD).

Effects of ethanol on the metabolism in vitro of trichloroethylene (TRI) and 1,1,1-trichloroethane (1,1,1-TRI)

Group	Microsomal protein (mg/g liver)	Cytochrome P-450 (nmol/mg protein)	Rate of metabolism (nmol/mg protein/min)	
			TRI	1,1,1-TRI
Control	17.2(1.6)	0.69(0.07)	0.82(0.21)	0.04(0.02)
Ethanol	20.6(1.9)*	0.88(0.07)**	4.48(0.15)**	0.19(0.04)**

Values represent the mean (SD) for five rats.
*p < 0.05; **p < 0.01 v Control.

Means were tested by the Student-Newman-Keuls' test.²² The 0.05 level of probability was chosen as the criterion of significance.

Results

TRI AND 1,1,1-TRI METABOLISM IN VITRO
Ethanol consumed in combination with a low carbohydrate and high fat diet increased liver

microsomal protein and cytochrome P-450 contents (table). The ethanol treatment enhanced both TRI and 1,1,1-TRI metabolism in vitro about fivefold. The rate of metabolism of 1,1,1-TRI was about 1/20 that of TRI when measured at almost the same substrate concentration of 0.1 mM.

TRI AND 1,1,1-TRI KINETICS AFTER INHALATION EXPOSURE

When rats were exposed to TRI at a concentration of 50 or 100 ppm the three week ethanol consumption, which increased the hepatic metabolism of TRI in vitro about five fold, had no influence on the kinetics of TRI: no significant difference was found between control and ethanol treated rats either in the TRI concentration in the blood or in the amounts of TCA and TCE excreted into the urine (figs 1 and 2). At an exposure level of

Figure 1 Trichloroethylene (TRI) concentration in blood (A); cumulative amounts of trichloroacetic acid (TCA) (B); and trichloroethanol (TCE) (C) excreted into urine after exposure to 50 ppm of TRI. Vertical lines depict SD (n = 5).

Figure 2 Trichloroethylene (TRI) concentration in blood (A); cumulative amounts of trichloroacetic acid (TCA) (B); and trichloroethanol (TCE) (C) excreted into urine after exposure to 100 ppm of TRI. Vertical lines depict SD (n = 5).

Figure 3 Trichloroethylene (TRI) concentration in blood (A); cumulative amounts of trichloroacetic acid (TCA) (B); and trichloroethanol (TCE) (C) excreted into urine after exposure to 500 ppm of TRI. Vertical lines depict SD ($n = 5$).

500 ppm the ethanol induced enhancement of TRI metabolism in vitro made its appearance in the TRI metabolism in vivo but the effect was only marginal with an increase of urinary excretion of TCA alone (fig 3). Ethanol consumption had no effect on either the TRI concentration in the blood or the amount of TCE excreted in the urine at this level of exposure. When the exposure was raised to 1000 ppm, however, ethanol consumption definitely enhanced the TRI metabolism in vivo as shown by a significant decrease in the blood TRI concentration as well as by a significant increase of both TCA and TCE excretion in the urine of the ethanol treated rats (fig 4). These findings suggest that enzyme induction significantly affects the pharmacokinetics of TRI only when the exposure is 500 ppm or higher.

Figure 4 Trichloroethylene (TRI) concentration in blood (A); cumulative amounts of trichloroacetic acid (TCA) (B); and trichloroethanol (TCE) (C) excreted into urine after exposure to 1000 ppm of TRI. Vertical lines depict SD ($n = 5$).

By contrast, in the case of 1,1,1-TRI, ethanol treated rats excreted more TCA and TCE in the urine than did the control rats at any exposure level studied (figs 5–7). The induction of enzymes however was not reflected in the concentration of 1,1,1-TRI in the blood at any level of exposure. These findings indicate that the effect of induction of enzymes on the pharmacokinetics of 1,1,1-TRI sharply contrasts with that of TRI.

Discussion

A three week ethanol consumption enhanced the hepatic metabolism of TRI and 1,1,1-TRI in vitro about five fold (table), almost to the same extent as that previously reported.⁸ The metabolism study in vitro also indicates that 1,1,1-TRI is poorly metabolised by the

Figure 5 1,1,1-Trichloroethane (1,1,1-TRI) concentration in blood (A); cumulative amounts of trichloroacetic acid (TCA) (B); and trichloroethanol (TCE) (C) excreted into urine after exposure to 50 ppm of 1,1,1-TRI. Vertical lines depict SD ($n = 5$).

Figure 6 1,1,1-Trichloroethane (1,1,1-TRI) concentration in blood (A); cumulative amounts of trichloroacetic acid (TCA) (B); and trichloroethanol (TCE) (C) excreted into urine after exposure to 100 ppm of 1,1,1-TRI. Vertical lines depict SD ($n = 5$).

hepatic microsomal enzymes, by contrast with TR, which is a highly metabolised substrate.¹²

Enzymes induced by ethanol consumption had an impact on the metabolism of TRI in vivo only at high exposure (500 ppm or higher; (figs 1–4), a finding that is in good agreement with previous experimental evidence from *m*-xylene, another highly metabolised substrate.²³ These findings together support the working hypothesis that the effect of induction of enzymes on the metabolism of some inhaled chemicals depends on the level of exposure and that the induction of enzymes or the enhancement of metabolism found in vitro is reflected in vivo only at a high level of exposure.¹¹

By contrast, enzymes induced by ethanol considerably affected the metabolism of 1,1,1-TRI in vivo at any exposure level from

50 to 500 ppm, although it had no influence on the blood concentration of the parent compound (figs 5–7). The reason why the enhancement of metabolism was not reflected in the blood 1,1,1-TRI concentration is that the clearance of 1,1,1-TRI by metabolism normally accounts for only a few per cent of the total elimination from the body, contrasting with that of TRI, which accounts for 75%.¹

Clearance of foreign chemicals by hepatic metabolism depends on the relation between the intrinsic metabolic clearance (V_{max}/K_m) and the hepatic blood flow (Q_H), and the metabolic clearance of foreign chemicals is often rate limited by Q_H independently of enzyme capacity (V_{max}) when the exposure concentration is low (perfusion limited metabolism).²⁴ Compounds with V_{max}/K_m larger than Q_H are highly metabolised

Figure 7 1,1,1-Trichloroethane (1,1,1-TRI) concentration in blood (A); cumulative amounts of trichloroacetic acid (TCA) (B); and trichloroethanol (TCE) (C) excreted into urine after exposure to 500 ppm of 1,1,1-TRI. Vertical lines depict SD ($n = 5$).

substrates.¹² These compounds are almost completely metabolised while passing through the liver as long as the concentration in the hepatic tissue (CH) is low. Thus the blood flow (QH) limits the extent of their metabolism and enzyme induction (V_{max} is increased) has little or no effect on the metabolism, provided that the exposure concentration is low.

When the concentration in the inhaled air is raised so that the metabolic clearance expressed in terms of $V_{\text{max}}/(K_m + CH)$ becomes smaller than QH, the V_{max} becomes a factor limiting the metabolism. In other words, the enzyme induction found in an in vitro metabolism study would be fully reflected in the in vivo metabolism only at high exposure concentrations where the metabolism is solely dependent on the capacity of enzymes.

On the other hand, for those solvents whose V_{max}/K_m is smaller than QH (poorly metabolised substrates) the enzyme activity limits the metabolism independently of the

level of exposure (capacity limited metabolism), and induction of enzymes can increase their metabolism in vivo even at a low level of exposure.¹²

Although the V_{max}/K_m values are not available for TRI and 1,1,1-TRI metabolism, our study indicates that the V_{max}/K_m of TRI is larger than QH and thus the perfusion limited metabolism prevails when the exposure remains below 100 ppm, whereas the V_{max}/K_m of 1,1,1-TRI is smaller than QH and thus the capacity limited metabolism is a normal occurrence independently of level of exposure.

Experimental evidence has been published indicating that induction of enzymes does not always produce a significant effect on the metabolism of inhaled chemicals in a living body. Phenobarbitone is another potent inducer of hepatic cytochrome P-450. Treatment of rats with phenobarbitone accelerated in vivo metabolism of a variety of organic solvents such as *m*-xylene,²⁵ benzene,^{26, 27} toluene,²⁶ and *n*-hexane,²⁸ but only at high doses of these solvents.

Phenobarbitone inducible cytochrome P-450 isozymes (primarily, P-450IIB1) have high K_m values for organic solvents, by contrast with ethanol inducible P-450IIE1, which is a low K_m enzyme for these compounds.^{6, 29} Our present findings together with the other reports mentioned earlier indicate that enzymes induced by ethanol (a typical inducer of low K_m isozymes) or phenobarbitone (a typical inducer of high K_m isozymes) will significantly affect the pharmacokinetics of highly metabolised organic solvents only when the exposure concentration is high.

In conclusion, enzyme induction by ethanol consumption may differently affect the pharmacokinetics of TRI and 1,1,1-TRI in human occupational exposure: TRI metabolism may be increased only at an exposure much higher than the current occupational exposure limit (mostly 50 ppm), whereas 1,1,1-TRI metabolism may be increased at an exposure similar to occupational exposure.

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