EFFECT OF ETHANOL ON UPTAKE OF TRICHLOROETHYLENE IN ISOLATED PERFUSED RAT LIVER

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Abstract.....The effect of ethanol on uptake of trichloroethylene in isolated perfused rat liver was investigated. The uptake of trichloroethylene was measured in both phenobarbital (PB) non-treated and PB treated rat livers. Furthermore, for PB treated rat livers, the fluorescence of reduced pyridine nucleotides, oxygen consumption, and scanning reflectance spectrum were measured in the liver, perfused with Krebs-Henseleit buffer saturated with a 92%O₂-5%CO₂-3%CO gas mixture. The uptake of trichloroethylene was decreased by 6.0% in the PB non-treated rat liver and 10.6% in the PB treated rat liver following the addition of ethanol. This uptake decrease was thought to arise mainly from the inhibitory effect of ethanol on mixed-function oxidation in the liver because of the corresponding decrease in oxygen consumption and absorbance difference $\Delta A_{450-490nm}$. The inhibition was considered to be due to interference with electron transfer to the complex of substrate and cytochrome P-450. Increase in intracellular NADH might also affect the formation of trichloroacetic acid since the reduction of NADH in the cytosol attained a maximum with 20 mM ethanol.

Key words: Trichloroethylene, Ethanol, Mixed-function oxidation, Perfused rat liver, Cytochrome P-450

INTRODUCTION

It is not uncommon for industrial workers to drink after undertaking work dealing with vapour solvents. Since trichloroethylene (Tri) is widely used industrially as a degreasing agent, we consider it important to clarify the interaction between Tri and ethanol. Several authors have described the inhibitory effect of ethanol on Tri metabolism in humans (Müller, et al., 1973; Windemuller & Ettema, 1978) and animals (White & Carlson, 1981). Also ethanol is known to inhibit mixed-function oxidation in the liver (Rubin, et al., 1970; Cinti, et al., 1973; Reinke, et al., 1980). In the present study, we examined the effect of ethanol on uptake of Tri by measuring the rate of Tri

uptake, fluorescence of reduced pyridine nucleotides, scanning reflectance spectrum, and oxygen consumption in isolated perfused rat liver.

MATERIALS AND METHODS

Animals

Male albino rats of Wistar strain, maintained on a commercial diet, were used. The animals were subdivided into two groups: a phenobarbital (PB) non-treated group and a PB treated group. Sodium phenobarbital (70 mg/kg body weight) was injected intraperitoneally, daily for 4 days.

Liver perfusion system

The perfusion technique was the same as that described elsewhere (Miller, 1973; Sugano, et al., 1978). The liver was perfused at 32°C with Krebs-Henseleit bicarbonate buffer, pH 7.4, saturated with oxygen: carbon dioxide (95: 5) in a non-recirculating system. The perfusion fluid passed through the liver via the vena portae and vena cava superior. Tri and ethanol were dissolved in the buffer and infused into the perfusion fluid entering the liver at final concentrations of 50 μ M and 20 mM, respectively. Surface fluorometry was carried out to observe the oxidation-reduction state of pyridine nucleotides (Chance, et al., 1962; Scholz, et al., 1970; Takano, 1979). The fluorescence of the reduced pyridine nucleotides was excited at 366 nm and monitored at 466 nm. Scanning reflectance spectrophotometry was carried out to observe the reflectance spectrum (Sies & Brauser, 1970; Iyanagi, et al., 1981). A scanning reflectance spectrophotometer was used to record the absorbance difference between the surface of the liver and reflectance (magnesium oxide plate), in the region from 420 nm to 700 nm. The triplex of substrate, cytochrome P-450 and CO was monitored as described Sies and Brauser (1970), using a gas mixture of O₂: CO₂: CO (92: 5: 3). The oxygen consumption in the effluent perfusion fluid was monitored with Clark-type oxygen electrode. The rates of oxygen consumption were expressed as nmoles/g liver/min.

Tri uptake by the liver

Samples of the perfusion fluid were collected for 10 seconds at every 7 minutes, and the concentration of Tri in each sample was determined by gas chromatography. The rates of Tri uptake was calculated from the arterio-venous concentration difference, and expressed as nmoles/g liver/min.

RESULTS

The uptake of Tri and its decrease with 20 mM ethanol are shown in Table 1. The uptake of Tri was decreased by 10.6% in PB treated rat livers and 6.0% in PB non-treated rat livers, respectively, by the addition of ethanol. The uptake of Tri achieved a steady state in 14 minutes. When the ethanol was discontinued, the uptake of Tri returned to their basal levels (Fig. 1).

Typical changes in oxygen consumption, pyridine nucleotide fluorescence and scanning reflectance spectra following the addition of 20 mM ethanol are shown in Fig. 2. The mean increase in oxygen consumption rates during Tri infusion were 122 nmoles/g liver/min, and the ratio of oxygen consumption increase to the Tri uptake was 1.14. The oxygen consumption during Tri infusion was decreased by 12.0% by the addition of ethanol and this decrease corresponded closely to the decrease in Tri uptake (Fig. 3).

The fluorescence of reduced pyridine nucleotides was decreased by 21.7% when Tri was infused and increased by 58.4% when 20 mM ethanol was subsequently infused. The increase in reduced pyridine nucleotides caused by ethanol in the absence of Tri was 56.8%.

Table 1. Trichloroethylene uptake by the liver

(nmoles/g liver/min)

* : Significantly different from PB non-treated group, $p < 0.01$. Mean $\pm$ S. D. are shown.

	Trichloroethylene 50 μ M	Trichloroethylene 50 μ M + Ethanol 20 mM
PB non-treated group (n = 10)	66.4 $\pm$ 10.1	62.4 $\pm$ 11.9
PB-treated group (n = 10)	107.0 $\pm$ 13.6*	95.8 $\pm$ 11.2*

Fig. 1. Effect of ethanol on trichloroethylene uptake by the liver in PB treated rats. Each value represents the mean $\pm$ S. D. (n = 10)

Fig. 2. Effect of ethanol on oxygen consumption, surface fluorescence, and absorbance differences in perfused rat liver.

Fig. 3. % changes in trichloroethylene uptake, oxygen consumption and absorbance differences caused by addition of 20 mM ethanol in perfused rat livers. (n = 10)

The spectral changes caused by Tri infusion revealed the triplex of substrate, cytochrome P-450 and CO ($\Delta A_{450-490 \text{ nm}}$) and the complex of substrate and cytochrome P-450 ($\Delta A_{576-600 \text{ nm}}$). In the presence of ethanol, the absorbance difference, $\Delta A_{450-490 \text{ nm}}$, was decreased as shown in Fig. 2. $\Delta A_{640-660 \text{ nm}}$, which reflects the spectral change caused by catalase- H_2O_2 intermediate, was increased. The decrease in $\Delta A_{450-490 \text{ nm}}$ was due partly to the change in catalase- H_2O_2 intermediate since 20 mM ethanol alone caused a slight decrease in $\Delta A_{450-490 \text{ nm}}$. The decrease in $\Delta A_{450-490 \text{ nm}}$ when this interference was cancelled out was 10.1% (Fig. 3). $\Delta A_{576-600 \text{ nm}}$ did not change significantly with ethanol.

DISCUSSION

Ethanol decreased the Tri uptake of isolated perfused livers. There are two possible explanations for this uptake decrease of Tri: an inhibitory effect on mixed-function oxidation and on the formation of trichloroacetic acid from chloral hydrate.

The inhibitory effect of ethanol on mixed-function oxidation was assumed from the present investigation. First, the uptake decrease of Tri was greater in PB treated rat livers than PB non-treated rat livers. Second, the oxygen consumption during Tri metabolism was decreased by ethanol and the decrease in oxygen consumption corresponded to the decrease in Tri uptake. Third, $\Delta A_{450-490 \text{ nm}}$ was decreased by ethanol, and this was thought to be due to a decrease of triplex of substrate, cytochrome P-450 and CO.

It has been proposed that ethanol inhibits mixed-function oxidation by competing with substrate for binding site (Rubin, et al., 1970; Cinti, et al., 1973), by decreasing the activity of cytochrome P-450 reductase (Rubin, et al., 1970), and by diminishing the generation of NADPH (Reinke, et al., 1980). $\Delta A_{576-600 \text{ nm}}$, which is thought to be a typical changes for observing the complex of substrate and cytochrome P-450 (Iyanagi, et al. 1981), was not changed significantly by ethanol. This suggests that the binding of substrate and cytochrome P-450 was not affected by ethanol in the isolated perfused liver. We considered, therefore, that the inhibition of mixed-function oxidation might be due to interference with electron transfer to the complex of substrate and cytochrome P-450 in the isolated perfused liver, which was consistent with the results of the *in vitro* experiment reported by Rubin et al. (1970).

Ethanol may also inhibit Tri metabolism by increasing intracellular NADH. It is well known that Tri is metabolized via an unstable epoxide to chloral hydrate by mixed-function oxidases which was converted to trichloroethanol and trichloroacetic acid. The formation of trichloroethanol from chloral hydrate requires alcohol dehydrogenase and NADH, while that of trichloroacetic acid requires chloral hydrate dehydrogenase and NAD (Reynolds & Moslen, 1979). It has been reported that simultaneous administration of Tri and ethanol decreased the concentrations of trichloroethanol and trichloroacetic acid in the blood and urine (Müller, et al., 1975; White & Carlson, 1981). The decrease in NAD/NADH caused by ethanol oxidation is thought to exert an in-

hibitory effect on the formation of trichloroacetic acid probably due to the lack of NAD. Conversely, ethanol oxidation was slightly inhibited due to competition between chloral hydrate and ethanol for alcohol dehydrogenase (Müller, et al., 1975). Lindros et al. (1974) has reported that the reduction of cytosolic pyridine nucleotides caused by 20 mM ethanol approaches a maximum. In the present investigation, the reduction of pyridine nucleotides caused by 20 mM ethanol during Tri infusion was 58.3%, and this value was considered to indicate a maximal reduction of pyridine nucleotides in the cytosol, which was responsible for the decreased formation of trichloroacetic acid.

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