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Jolanta Jajte[1], Jan Stetkiewicz[2], Teresa Wrońska-Nofer[3]

OXIDATIVE STATUS EVALUATED IN RATS EXPOSED TO 1-BUTANOL AND ETHANOL

[1]Department of Toxicology, Medical University of Łódź, Łódź, Poland
[2]Department of Pathomorphology, [3]Department of Toxicology and Carcinogenesis
Nofer Institute of Occupational Medicine, Łódź, Poland

ABSTRACT

Introduction: The study focused on the evaluation of the pro-oxidative effect of acute ingestion of ethanol superimposed on prolonged 1-butanol vapour inhalation to simulate combined exposure to both alcohols in the industry: short term ethanol abuse and long-term exposure to 1-butanol.

Material and Methods: Experiments were performed on male Wistar rats exposed to 1-butanol at the concentration of 320 mg/m^3 for 3 months (5 h/day, 5 days/week), in dynamic inhalation chamber) and to ethanol (6 oral doses, 0.25 g/100 g b.w.) administered at 12 h intervals for the last three days of exposure). To estimate the redox state, cytochrome P-450 level, glutathione sulfhydryls content, glutathione-S-transferase activity, microsomal lipid peroxidation rate in the liver, and the total antioxidant potential of the serum, were determined.

Results: The studies revealed that the combined exposure to ethanol and 1-butanol, contrary to exposure to 1-butanol vapour only or ethanol ingestion, resulted in the stimulation of hepatic microsomal lipid peroxidation with inhibition of glutathione-S-transferase activity, as well as inhibition of serum antioxidant potential.

Conclusions: Disturbances of the oxidative status induced by combined exposure may contribute to disorders in the lipoprotein structure of biomembranes both at subcellular and cellular levels with special reference to target organ, and thus aggravate the biological consequences of ethanol abuse and occupational exposure to 1-butanol.

Key words: 1-butanol, ethanol, lipid peroxidation, glutathione-S-transferase

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Address for correspondence:
Jan Stetkiewicz
Nofer Institute of Occupational Medicine
St. Teresy 8
90–950 Łódź, Poland
Phone: 48 42 631–46–39
E-mail: janstet@imp.lodz.pl

INTRODUCTION

Health risk of the combined effect of ethanol and 1-butanol results from occupational exposure to 1-butanol vapours and occasional ethanol abuse, and consumption of commercial alcoholic drinks containing higher chain alcohols (e.g., izomers of butanol) that can be induced in addition to ethanol [1].

Toxicokinetic interactions of combined exposure to ethanol and butanol arise from similar enzymatic pathways involved in their metabolism. Both alcohols are metabolised with the contribution of cytochrome P-450 monooxygenases and alcohol dehydrogenase. Thus cytochrome P-450 monooxygenases may be induced by both alcohols. Alcohol-inducible cytochrome P-450 is able to produce reactive oxygen species (ROS) and through this mechanism may contribute to xenobiotic activation, eg. 1-hydroxybutyl radicals were identified in the rat liver microsomes incubated with 1-butanol in the presence of NADPH [2]. It has also been demonstrated that oxidation of 1-butanol in the liver microsomes contributes to ethanol-inducible cytochrome P-450 2E1. Cytochrome P-450, through generation of free radicals becomes the major enzyme responsible for the activation of alcohols in ethanol/butanol-fed rats [3,4]. Moreover, it was indicated that the capacity of cytochrome P-450 2E1 to reduce oxygen is related to its ability to generate alcohol free radicals and that ferric cytochrome P-450-oxygen complex might act an oxidizing species toward other alcohols [2]. In this study, it was assumed that joint exposure to ethanol and 1-butanol may intensify derangement of the redox state in the target organ of the rats induced by each of these alcohols separately. Therefore, we decided to investigate the pro-oxidative effect of acute ingestion of ethanol superimposed on prolonged 1-butanol vapours inhalation in rats. Experimental model applied in the study simulates the way of combined exposure to both alcohols in the industrial environment: chronic occupational exposure to 1-butanol and occasional ethanol abuse.

MATERIALS AND METHODS

Treatment

Experiments were carried out on male Wistar rats (outbred IMP:WIST) of 200–250 g/b.w., fed with a laboratory diet – Murigram (Agropol, Motycz, Poland) with free access to water.

Rats were divided into four groups:

— Control group — rats were kept in dynamic inhalation chamber for 3 months (5 h daily, 5 days weekly),
— Ethanol group — rats were kept in dynamic inhalation chamber for 3 months (5 h daily, 5 days weekly) and for the last 3 days were treated with ethanol. Ethanol (reactified Spirit, Polmos, Poland) was administered by gavage (6 oral doses of 0.25 g/100 g b.w. at 12 h intervals),
— 1-Butanol group — rats were exposed for 3 months (5 h daily, 5 days weekly) to 1-Butanol (POCH, Poland) at a concentration of 320 mg/m^3 in dynamic inhalation chamber,
— 1-Butanol + Ethanol group — rats were exposed for 3 months (5 h daily, 5 days weekly) to 1-Butanol (POCH, Poland) at a concentration of 320 mg/m^3 in dynamic inhalation chamber and for the last 3 days of exposure jointly treated with ethanol (6 oral doses of 0.25 g/100 g b.w. at 12 h intervals).

Each group consisted of 6 animals.

Concentration of 1-butanol vapours in the chamber was controlled by means of gas chromatograph (Varian 1440).

In all experiments the Polish law on the protection of animals was followed [5].

Preparation procedures

Rats, fasted overnight, were sacrificed by decapitation and the livers and blood were immediately processed. Livers were excised and homogenized with three volumes of KCl (150 mmol) buffered with Tris-HCl (10 mmol), pH 7.4 in a glass Potter-Elvehjem homogenizer with a teflon pestle to yield a 10% (w/v) homogenate. Microsomes and cytosol were obtained from the postmitochondrial supernatant by ultracentrifugation at 105 000 g at 4°C for 75 min in Beckman XL-70 OPTIMA preparative ultracentrifuge.

Biochemical assays

Glutathione sulfhydryls (GSH) was determined by the method of Ellman et al. [6] and glutathione-S-transferase (E.C. 2.5.1.18) by the method of Habig et al. [7]. Cytochrome P-450 in microsomes was determined according to Omura and Sato [8]. Carbon monoxide difference spectra of dithionite-reduced microsomes were charted between 490 and 450 nm using Beckman ACTA CIII spectrophotometer. An extinction coefficient of 91 mmol^{-1} cm^{-1} was used for cytochrome P-450 quantifying. Protein was determined according to Lowry et al. [9]. Lipid peroxidation in the fresh microsomal *(in vivo)* was

evaluated on the basis of the detection of thiobarbituric acid reactive substances (TBARs), as malondialdehyde (MDA), according to Mihara et al. [10]. An extinction coefficient of 156 000 $mol^{-1}cm^{-1}$ according to Wills was used for MDA formation [11]. The antioxidant activity of serum (AOA) was evaluated using ox-brain homogenate according to Stocks et al. [12]. MDA concentrations in the zero time and after 1 h were measured by the thiobarbituric acid reaction. The AOA of serum was expressed in terms of percentage inhibition of spontaneous auto-oxidation.

Statistical analysis

For comparisons of the exposed group to control group and ethanol group the Student`s t-test was used. The differences were considered statistically significant at $p < 0.05$.

RESULTS

General effects of exposure

Exposure to 1-butanol vapours did not influence the food consumption or body weight gain, which was similar in all groups to and amounted to 0.75 g per day. No statistically significant differences were found in the rat body weight, liver wet weight, protein contents of the liver microsomes or glutathione sulfhydryls (GSH) level between the control and exposed groups (Tab. 1 and 2).

Effects of ethanol

Acute administration of ethanol to rats exerts stimulatory effect on the lipid peroxidation in microsomal fraction, but the indicated differences were not statistically significant in comparison with the control group (Tab. 3).

Table 1. Effects of 1-butanol inhalation and/or acute ethanol administration on cytochrome P-450 level in the liver of rats

Group	Microsomal protein [mg/g liver]	Cytochrome P-450 [nmol/mg microsomal protein]
Control	22.8±0.80	0.79±0.03
Ethanol	21.3±0.44	0.87±0.05
1-Butanol	23.9±0.86	1.00±0.05[a]
1-Butanol+Ethanol	21.7±0.70	0.82±0.03

Groups: Control — given tap water ad libitum; Ethanol — 6 oral doses of 0.25 g/100 g body weight in 12 hours intervals for 3 days; 1-Butanol — inhalation exposure to 1-butanol at concentration of 320 mg/m^3, 5 h daily for 3 months; Ethanol+1-Butanol — at the end of exposure to 1-butanol — for 3 days — jointly administered ethanol (5 oral doses of 0.25 g/100 g body wt for 3 days).
Each group consisted of 6 animals. Results are the mean ±SE.
[a] Significantly different from the control at $p < 0.05$.

Table 2. Changes in the activity of glutathione-S-transferase and glutathione-SH content in the liver of rats chronically exposed to 1-butanol and/or ethanol

Group	Glutathione-SH [μmol/g tissue]	Glutathione--S-transferase [μmol/g tissue/min]
Control	6.48±0.31	68.20±1.21
Ethanol	6.60±0.31	60.10±2.77
1-Butanol	6.64±0.39	65.09±1.60
1-Butanol+Ethanol	6.12±0.29	57.20±1.50[a]

Details are described in Table 1.

Table 3. The rate of lipid peroxide formation in the liver microsomal fraction in control and exposed animals and serum antioxidant activity

Group	Lipid peroxidation *in vivo* [nmol MDA/mg] microsomal protein	Antioxidant activity in serum per cent
Control	1.23±0.09	23.0±0.9
Ethanol	2.27±0.65	18.7±2.0
1-Butanol	1.30±0.19	17.5±1.7
1-Butanol+Ethanol	3.10±0.56[a]	15.3±1.5[a]

Details are described in Table 1.
MDA — malondialdehyde.

Effects of 1-butanol

1-Butanol vapours evoked neither statistically significant changes in the hepatic gluthatione-S-transferase activity (Tab. 2) nor lipid peroxidation in microsomal fraction (Tab. 3). Prolonged exposure to 1-butanol induced cytochrome P-450 in the liver microsomes, $p < 0.05$ (Tab. 1).

Effects of combined exposure to 1-butanol and ethanol

Ingestion of ethanol superimposed on prolonged 3-month inhalation of 1-butanol vapours provoked statistically significant peroxidation of microsomal lipids ($p < 0.05$) in the liver and the decrease of antioxidant serum reserve. This increase was concomitant with slight but significant decrease in the glutathione-S-transferase activity (Tab. 2). The biotransformation capacity of the liver, as inferred from the level of cytochrome P-450, remained unchanged (Tab. 1). However, the ultrastructural examination of the hepatocytes performed in this study showed moderate proliferation of smooth endoplasmic reticulum, the source of cytochrome P-450 monooxygenases.

DISCUSSION

The results of the study indicated that in rats exposed to 1-butanol at concentration of 320 mg/m^3, lipid peroxidation and glutathione sulfhydryls content as well as glutathione-S-transferase activity were unaffected. However, a combined short-term exposure to ethanol superimposed on a long-term exposure to 1-butanol vapours brought about a biological effect, manifested by enhanced lipid peroxidation and decreased glutathione-S-transferase activity in the liver. This increase was concomitant with a statistically significant decrease in serum antioxidant potential.

Oxidant status in the liver may be considered as a consequence of two opposing events: 1 — generation of free radicals, e.g. in the course of cytochrome P-450 catalysed hydroxylation of xenobiotics (organic solvents), and 2 — reduction of antioxidant potential, free radical-scavenging enzymes (the biological self-protecting system) [13,14,15]. Data obtained in this study demonstrated that disturbances in the liver oxidant status provoked by a combined exposure to 1-butanol and ethanol may be categorized into direct pro-oxidative effect and reduction of antioxidative reserve.

Over the last years, it has been suggested that lipid peroxidation might play an important role in the liver damage induced by ethanol. An increased level of lipid peroxides was demonstrated in the liver of ethanol abuse persons and animals acutely and chronically treated with ethanol [16,17,18,19]. Comporti et al. [20] demonstrated changes in the hepatic membrane fatty acids due to acute ethanol administration. It has been found that short-chain alcohols (ethanol, methanol and n-butanol) interact with lipid membranes, and replace water bound at this site. Through this mechanism alcohol may cause membrane destabilisation and thus promote lipid peroxidation [21] A quantitative immunohistochemical analysis indicated fivefold increase of malondialdehyde and 4-hydroxynonenal in the liver of rats treated with alcohol [22]. It has also been suggested that ethanol-induced lipid peroxidation seems to be the consequence of impaired hepatocellular glutathione-dependent defense system (glutathione and glutathione-related enzymes) [23,24].

The assumption that ethanol, as a pro-oxidative factor, may influence the metabolic and oxidative effect of 1-butanol, a toxic chemical occurred in the industrial environment, was partly evidenced: ethanol enhance the 1-butanol mediated increment of microsomal lipid peroxidation in the liver and decrease the antioxidant potential of the serum although it failed to increase metabolic capacity of the liver. However, these changes may contribute to disorders in the lipoprotein structure of biomembranes, both at subcellular and cellular levels, with special reference to the target organ/system (microsomal membranes, vessel walls) and a finally may potentiate the biological consequences of occupational exposure to 1-butanol and ethanol abuse.

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J. Jajte, J. Stetkiewicz, T. Wrońska-Nofer

STRES OKSYDACYJNY U SZCZURÓW EKSPONOWANYCH NA 1-BUTANOL I ETANOL

Streszczenie

Szczury, samce rasy Wistar narażano inhalacyjnie na pary 1-butanolu w stężeniu 320 mg/m^3 przez 3 miesiące, pięć godzin dziennie. Części zwierząt w ostatnich trzech dniach ekspozycji inhalacyjnej podano *per os* etanol w dawce 0,25 g/100 g masy ciała, 6 razy co 12 godzin. W celu określenia statusu oksydacyjnego oznaczano w wątrobie poziom cytochromu P-450, aktywność S-transferazy glutationowej, peroksydację lipidów oraz w surowicy — całkowity potencjał antyoksydacyjny.

Zarówno 1-butanol, jak i sam etanol nie spowodował istotnych zmian poziomu ocenianych wskaźników. Etanol wywołał jedynie nieistotny statystycznie 50% wzrost peroksydacji lipidów błon mikrozomalnych. Natomiast w warunkach łącznej ekspozycji na 1-butanol i etanol stwierdzono w wątrobie istotny wzrost stopnia peroksydacji lipidów błona mikrosomalnych, obniżenie aktywności transferazy glutationowej oraz obniżenie poziomu antyoksydacyjnego surowicy.

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