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Short communication

Interaction between tobacco smoke and alcohol in animal models

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Abstract:
This study aimed to evaluate the impact of nicotine and other xenobiotics contained in tobacco smoke on the pharmacokinetics of ethyl alcohol and on the levels of toxic ethanol metabolites such as acetic aldehyde. We also sought to evaluate the impact of a one-time administration of ethyl alcohol on the biotransformation of nicotine, the addictive alkaloid of tobacco smoke, to its main metabolite, cotinine.
Rats were divided in three groups. The first group was exposed to tobacco smoke (6 h per day, for 5 days), the second group was treated with alcohol (2 g/kg), and the third group was exposed to tobacco smoke and treated with alcohol.
Earlier exposure to tobacco smoke had an insignificant impact on the elimination of alcohol, but caused a significant increase in the volume of distribution, which could be caused by an increase in the first-pass effect. In contrast, inhaling tobacco smoke decreased acetic aldehyde concentrations in the first hour after alcohol administration. The major finding of this study was that a single dose of ethyl alcohol increases the rate of elimination of cotinine, the major metabolite of nicotine. This was demonstrated by a reduced biological half-life ($t_{0.5}$) and mean resident time (MRT).

Key words:
tobacco smoke, ethyl alcohol, pharmacokinetics, interaction

Introduction

Tobacco smoke contains 4,300 chemical compounds, which may interact with drugs or other xenobiotics taken at the same time.

One predominant effect of smoking is the increased metabolism of drugs as a result of the induction of liver enzymes. The main enzyme (CYP1A2) inducers contained in tobacco smoke are polycyclic aromatic hydrocarbons. Tobacco increases the rate of biotrans-

formation of different drugs, leading to changes in their therapeutic action [16].

The enzyme primarily responsible for alcohol metabolism is cytoplasmic alcohol dehydrogenase; however, some alcohol is metabolized by the microsomal enzyme CYP2E1 [13]. This metabolic route has increased significance in people addicted to alcohol after ingesting large quantities [14].

CYP2E1 participates in the biotransformation of various components of tobacco smoke, including tobacco smoke-specific nitrosamines [20]. This cytochrome also metabolizes caffeine, which is frequently taken jointly with alcohol and tobacco [12].

The nicotine contained in tobacco smoke increases the activity of liver and brain CYP2E1 in rats; the intensity of the effect depends on the length of exposure [10]. Clinical studies revealed the possibility of an interaction between ethanol and tobacco smoke through the induction of CYP2E1 [7]. Apart from those compounds increasing the activity of drug-metabolizing enzymes, tobacco smoke also contains carbon monoxide, cadmium, pesticides, cyanides and acrolein, which can inhibit the activity of drug-metabolizing enzymes [15].

In the experiments conducted on animals, we also evaluated the interaction of ethyl alcohol with tobacco smoke condensate; the interactions of specific components of tobacco smoke such as nicotine on some brain receptors [3]; and damage to the gastric mucosa due to specific components of tobacco smoke [18]. Many studies have demonstrated that both cigarette smoking and ethyl alcohol consumption are related to gastric and duodenal ulcers in people and animals [9].

Epidemiological studies indicate that the combination of tobacco smoking and drinking alcohol increases the risk of laryngeal cancer [17]. However, there is a lack of evidence for a pharmacokinetic interaction between alcohol and tobacco smoke components. Few published studies described an interaction between nicotine and alcohol in alcoholic patients [2, 6]. The aim of this study was to evaluate the impact of nicotine and other xenobiotics contained in tobacco smoke on the pharmacokinetics of ethyl alcohol and on the levels of toxic ethanol metabolites such as acetic aldehyde. We also sought to evaluate the impact of a one-time administration of ethyl alcohol on the biotransformation of nicotine to its main metabolite, cotinine.

Materials and Methods

The protocol for this animal experiment was approved by the Local Ethics Commission for Animal Studies in Poznań (No. 02/2008, January 18th 2008).

Female Wistar rats (160 ± 10 g) bred at the Department of Toxicology, University of Medical Sciences' were housed in polycarbonate cages containing woodchip bedding. A standard pellet diet and water were available *ad libitum.* The 12/12 h light/dark cycle, temperature 20–22°C and humidity 50–60% were maintained throughout the study. After 14 days of acclimatization, the rats were randomized and divided to three groups of 21 animals each:

I – animals were exposed to tobacco smoke for 6 h per day, for 5 days. CO concentration was taken as an index of tobacco smoke concentration and was maintained at 1,500 mg CO/m^3. Rats were exposed in a dynamic toxicological chamber [4] to tobacco smoke generated from a Polish brand of cigarettes without a filter tip ("Poznańskie" – Imperial Tobacco Polska S.A.);

II – an alcohol solution (10%) at a dose of 2 g/kg was given by gavage;

III – animals were exposed to tobacco smoke for 6 h per day, for 5 days. Rats were exposed to a 10% alcohol solution at a dose of 2 g/kg (administered by gavage). CO concentration was taken as an index of tobacco smoke concentration and was maintained at 1,500 mg CO/m^3.

After exposure to tobacco smoke (group I) or administration of alcohol (groups II and III), animals were anesthetized (xylocaine 40 mg/kg and ketamine 5 mg/kg). Blood samples were collected at seven time-points (0.25, 0.5, 1, 2, 3, 5, 24 h) with three rats per point.

Cotinine measurement

Cotinine concentration was measured by an immunoenzymatic method (ELISA) with application reagents for Cotinine Direct Elisa from BioQuant. All determinations were performed according to the manufacturer's instructions. The linear range of determination was 5–500 μg/l of cotinine, the limit of detection was 1 μg/l, and the limit of determination was 5 μg/l. The inter- and intraday coefficients of variation for 5, 50, and 100 μg/l of cotinine were below 10%.

Ethyl alcohol measurement

Ethanol concentration was determined by REA (Radiative energy attenuation) using the AxSYM analyzer and Abbott reagents. All determinations were performed according to the manufacturer's instructions. The linear range of determination was 0.1–3 g/l of ethanol, the limit of detection was 0.052 g/l, and the limit of determination was 0.1 g/l. The inter- and intraday coefficients of variation for 0.5, 1, and 1.5 g/l of ethanol were below 10%.

Measurement of acetaldehyde, acetone, methanol, n-propanol and n-butanol

The concentrations of volatile compounds were determined by gas chromatography after headspace solid phase microextraction (MSPE), as previously described [8].

Microextraction was carried out using a Supelco device equipped with fibers of SPMS Fibre Assembly (65 microns, Carbowax/DVB type). During the extraction process, the samples underwent ultrasonic treatment and heating at 60°C for 5 min. Desorption followed in the sample injector of the chromatograph at 220°C for 2 min.

The separation and quantitative analysis of the studied compounds were carried out using the gas chromatography method. We used a gas chromatograph ATI UNICAM 610 Series with a flame-ionization detector (FID) equipped with capillary column GS-Q of 30 m length and ID = 0.53 mm, J&W Scientific. The temperature of the sample injector was 220°C and that of the detector was 240°C. The separation was performed according to the following temperature scheme: 80°C for 4 min.; heating to 180°C at 10°C/min; maintenance of the final temperature for 3 min.

The linear range of determinations were: acetone 0–40 mg/l, acetaldehyde 0–40 mg/l, methanol 0–500 mg/l, n-propanol 0–40 mg/l, and n-butanol 0–40 mg/l. The lower limits of detection were 1 mg/l for methanol and 0.5 mg/l for the other compounds determined; the lower limits of determination were 2 mg/l for methanol and 1 mg/l for other compounds.

The interday coefficient of variation for two concentrations (5 and 20 mg/l) for each compound ranged from 6.05% for n-propanol to 11.68% for acetaldehyde. The intraday coefficients of variation for all determined compounds were below 10%.

Statistical and pharmacokinetic analysis

All statistical calculations were carried out with the STATISTICA 6.0 computer program. Analysis of variance was determined by *post-hoc* Tukey HSD test. The homogeneity of variance was verified with the Levene test.

Pharmacokinetic analysis of cotinine was carried out by the model-independent method (statistical moment analysis) using the SPLINE computer program. The following parameters were calculated: area under the curve (AUC), area under the first moment curve (AUMC), and mean resident time (MRT = AUMC/AUC). The biological half-life of cotinine ($t_{0.5}$) was equal to ln2/k, where k is the elimination constant of cotinine, calculated from the terminal portion of cotinine elimination. The Widmark coefficient β_{60} (for ethanol) was calculated by the linear regression method using the concentrations of ethanol at the 2-, 3- and 5-h time-points. For calculating the volume of ethanol distribution, we applied the equation presented below:

$V_d = D \times AUMC/AUC^2$ (D – dose of ethanol).

Results and Discussion

Most studies concerning pharmacokinetic interactions between tobacco smoke and alcohol concern chronic exposure to these xenobiotics, whereas there are few studies indicating whether short-term exposure to tobacco smoke and alcohol lead to similar interactions.

On average, 72% of nicotine absorbed by an organism is metabolized to its main metabolite, cotinine; due to individual differences, this value may range from 55% to 92% [1]. Others have used cotinine concentration in blood and urine or saliva as a biomarker of tobacco smoke exposure [5].

In this study, cotinine concentration in blood serum was applied as the biomarker of tobacco smoke exposure. The profile of changes in the cotinine concentration in the serum of animals exposed to tobacco smoke (groups I and III) is presented in Figure 1; the calculated pharmacokinetic parameters are collected in Table 1.

The maximal concentration of cotinine in the group of animals exposed to tobacco smoke (Group I) was achieved 30 min after the end of exposure (114.42

mg/l). In the group of animals exposed to tobacco smoke and alcohol (group III), the maximal concentration was slightly lower and delayed for 1–2 h (Fig. 1). Although the time courses of cotinine blood concentrations were not significantly different between groups I and III, calculated pharmacokinetic parameters indicated that ethanol co-treatment enhanced cotinine elimination (Tab. 1).

Both the biological half-life and the MRT were significantly lower in the group of animals from group

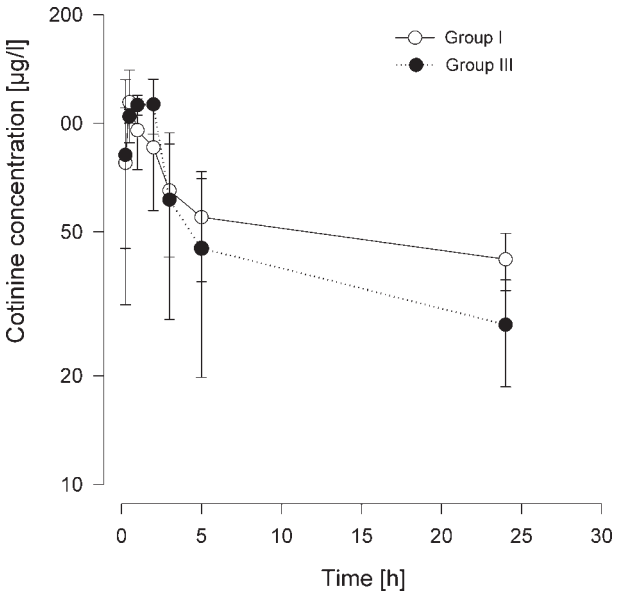

Fig. 1. Time course of the effect of tobacco smoke and ethyl alcohol administration on cotinine concentration in rat blood. Results are expressed as the mean ± SD for 3 animals per time-point. Group I – tobacco smoke, group III – tobacco smoke and ethanol administration

Tab. 1. Effects of tobacco smoke and ethyl alcohol on selected pharmacokinetic parameters of cotinine and ethanol in rat blood

Parameter	COTININE	
	Group I	Group III
AUC [h mg/l]	1834.53 ± 785.83	1603.20 ± 639.78
AUMC [mg h^2/l]	43358.97 ± 17735.40	32774.82 ± 12484.56
MRT [h]	23.86 ± 1.27	20.51 ± 1.13*
$t_{0.5}$ [h]	16.22 ± 1.16	13.50 ± 1.26*
Parameter	ETHANOL	
	Group II	Group III
AUC [h g/l]	5.00 ± 1.96	3.49 ± 0.60
V_d [l/kg]	0.74 ± 0.15	0.98 ± 0.08**
β_{60} [g/l h]	0.334 ± 0.069	0.276 ± 0.058

Data were calculated from experiments presented in Figure 1 and 2 and are the mean ± SD for 3 animals per time-point. AUC – area under the curve, concentration-time; AUMC – area under the first moment curve; MRT – mean resident time; $t_{0.5}$ – biological half-life, V_d – volume of distribution; β_{60} – Widmark coefficient; * $p < 0.05$ *vs.* group I; ** $p < 0.05$ *vs.* group II

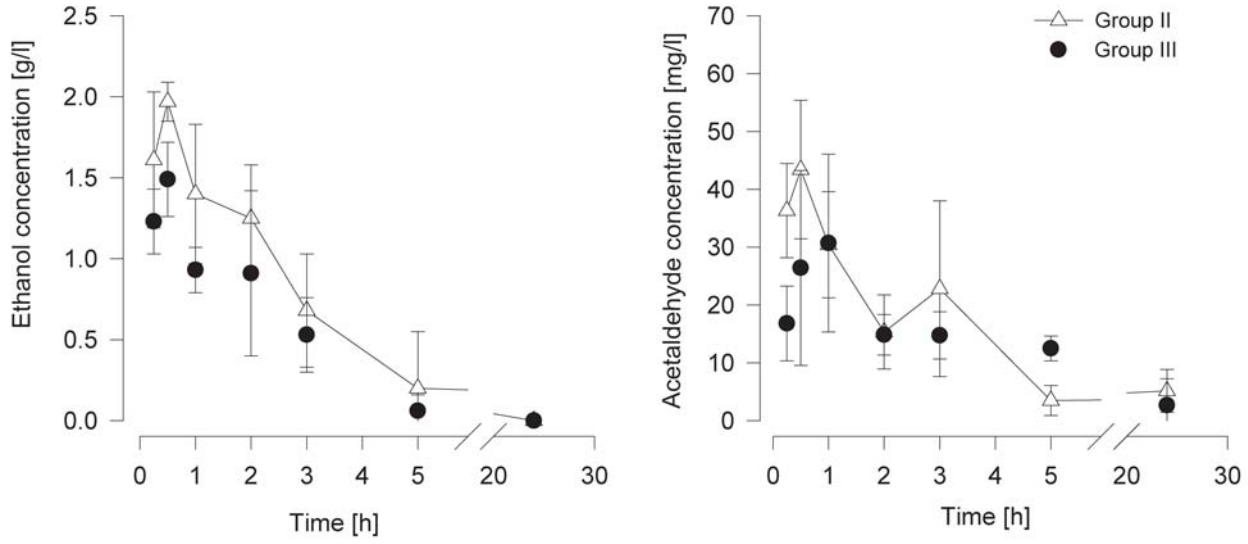

Fig. 2. Time course of the effect of tobacco smoke and ethyl alcohol administration on ethanol and acetaldehyde concentrations in rat blood. Results are expressed as the mean ± SD for 3 animals per time-point. Group II – ethanol, group III – tobacco smoke and ethanol administration

III in comparison with group I. On the basis of the obtained results, we cannot conclude whether a one-time administration of ethyl alcohol sped up the biotransformation of nicotine, or whether it affected the volume of distribution. The model of exposure applied in the studies (exposure to nicotine from tobacco smoke) does not allow researchers to calculate the exact dose of nicotine administered to a rat; consequently, the volume of distribution cannot be calculated accurately.

It is difficult to compare our data with those of previously published studies. To our knowledge, the only studies of this type were conducted on humans, presumably alcoholics, who had consumed alcohol for years [2, 6].

We measured the concentrations of ethyl alcohol, acetic aldehyde (Fig. 2), acetone, methyl alcohol, n-propyl alcohol, and n-butyl alcohol (data not shown) in blood samples from animals in all groups.

A maximal blood ethanol concentration of 1.97 g/l was demonstrated 30 min after administration in animals that received only ethanol (group II). This value was slightly (non-significantly) higher than in animals that received tobacco smoke plus ethanol (group III), which displayed an average ethanol concentration of 1.49 g/l (Fig. 2). These effects were followed by a gradual decrease in ethanol concentrations, reaching approximately zero at 5 h after administration in both groups (Fig. 2).

The calculated area under the curve for ethyl alcohol concentration – time (AUC) in the case of animals from the second group amounted to 5.00 ± 1.96 h g/l and was higher than in the case of animals from group III (3.49 ± 0.60 h g/l), but these differences were not statistically significant (Tab. 1). Since no differences were demonstrated in the coefficient of elimination (β_{60}) of ethyl alcohol between these groups, the slight decrease in the AUC value could be a result of the first-pass effect, or could caused by an increase in the volume of distribution (Vd), which was significantly higher in group III than in group II (Tab. 1).

The main metabolite of ethyl alcohol produced in the presence of alcohol dehydrogenase and cytochrome CYP2E1 is acetaldehyde [21]. Acetic aldehyde was present in the blood of animals that were given ethyl alcohol (group II and III), but not in group I (Fig. 2). The maximal concentration (49.78 mg/l) of this metabolite was achieved at 30 min after ethanol administration in group II and was about 30% (although not significantly) higher than in the group exposed to alcohol and tobacco smoke (37.08 mg/l; 1 h) (Fig. 2). These results could suggest a decrease in the rate of metabolism of ethanol to acetic aldehyde as a consequence of prior exposure to tobacco smoke; however, such a hypothesis is not confirmed by the observed lack of changes in the rate of elimination of ethanol (Widmark coefficient).

Volatile organic compounds such as acetone, methyl alcohol, and isopropanol are abundant in the blood of alcohol-drinkers [22]. The compounds get into blood with the consumed alcohol, occurring there due to contamination, and can also be produced as a consequence of physiological processes [11, 19]. In all the studied groups, the concentrations of acetone, methyl alcohol, n-propyl alcohol and n-butyl alcohol changed similarly over time (data not shown).

We conclude that prior exposure to tobacco smoke has an insignificant impact on the elimination of alcohol, causing a significant increase only in the volume of distribution, which could result from an increase in the first-pass effect. However, the inhalation of tobacco smoke tended to decrease the concentration of acetic aldehyde during the first hour after alcohol administration. The exposure of laboratory animals to tobacco smoke seems to have an insignificant impact on the concentrations of other volatile organic compounds accompanying ethanol, such as methyl alcohol, n-propyl alcohol, n-butyl alcohol, and acetone.

The primary finding of this study was the demonstration that a single dose of ethyl alcohol increases the elimination rate of the major metabolite of nicotine, cotinine, as shown by reduced $t_{0.5}$ and MRT values. However, further investigation is required to elucidate the mechanisms underlying this phenomenon.

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