ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN-POLONIA
VOL. LXI, N1, 55 SECTIO D 2006

Department of Clinical Pathomorphology
Experimental Teratology Unit of the Human Anatomy Department
Pediatrics Pulmonology and Rheumatology Department
Jaw Orthopedics Department, Medical University of Lublin

JAROSŁAW DUDKA, FRANCISZEK BURDAN, MONIKA CHYŻYŃSKA, AGNIESZKA LEWIŃSKA, JUSTYNA SZUMIŁO, AGNIESZKA KOROBOWICZ, EDYTA TOKARSKA, LECH WRONECKI, ELŻBIETA KOROBOWICZ

Comparative evaluation of ethanol versus 4-methylpyrazole effect on plasma alanine and aspartate aminotransferase activity and total bilirubin level in methanol poisoning rat N_2O/O_2 model

Ethanol is a well known antidote in methanol and ethylene glycol poisoning that prevents the synthesis of toxic aldehydes and acids. Despite its numerous advantages, some side-effects might complicate ethanol therapy in methanol poisoning of which central nervous system depression, pancreatitis or hypoglycaemia seem to be the most important (5, 6, 8). The alternative compound with ethanol-like detoxication mechanism is 4-methylpyrazole (4-MP) that has been used in clinical treatment of methanol poisoning (9). It prevents methanol transformation to its primary metabolite – formaldehyde, by inhibition of alcohol dehydrogenase (ADH) activity.

Many *in vitro* and *in vivo* studies have been conducted to evaluate toxicity of ethanol and 4-MP alone or with methanol, but none of them has compared side-effects of ethanol and 4-MP in methanol poisoning. Our previous study has proved that in methanol poisoning, 4-MP normalizes blood pH value at doses of 15 and 50 mg/kg (2). The present study is focused on comparative evaluation of plasma aminotransferase activity and total bilirubin concentration level in methanol intoxicated rats treated with ethanol or 4-MP.

It should be stressed that rodents do not exhibit the methanol poisoning syndrome observed in primates. One of the ways to ensure similar response of rats to methanol, like that observed in humans, is based on the inhibition of synthetase methionine (EC 2.1.1.13) (3, 4). The well known inhibitor of the enzyme is nitrous oxide (N_2O). The inhibition of synthase methionine results in the decrease in folate equivalents in rat to the level found in humans. Consequently, oxidation of formate, which is the most toxic metabolite of methanol, is reduced. Therefore, the study was performed on rat model using the N_2O/O_2 gas mixture.

MATERIAL AND METHODS

The experiment was carried out on mature (180–220 g) male Wistar CRL: (WI) WUBR strain (Warsaw, Poland) in accordance with the Guide for the Care and Use of Laboratory Animals and fully approved by the Local Ethical Committee (461/2004). Animals were kept under standard laboratory condition as described before (1).

The animals were assigned randomly to one of experimental groups. The substances were administered as follows: control – group (saline); group I – N_2O/O_2; group II – N_2O/O_2, methanol 3 g/kg b.w.; group III – N_2O/O_2, methanol 3 g/kg b.w. + 4-MP 15 mg/kg b.w.; group IV – N_2O/O_2, 4-MP 50 mg/kg b.w.; group V - N_2O/O_2, methanol 3 g/kg b.w. + ethanol 0.5 g/kg b.w.; group VI – N_2O/O_2, methanol 3 g/kg b.w. + ethanol 1 g/kg b.w.

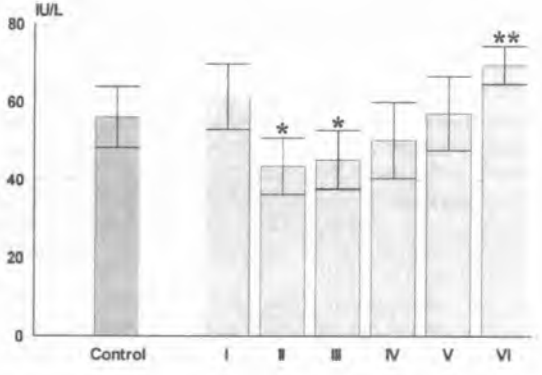
All rats, except for the control group, were placed in a plexiglass chamber (22x55x22 cm) and exposed to a mixture of N_2O/O_2 (1:1; flow rate 2 liters/min) for 4 h before the administration of methanol or saline. N_2O/O_2 exposure was continued throughout the study. Both gases (Linde Gaz Polska; Kraków, Poland) were for medical use. Methanol was administered intragastrically at a dose of 3 g/kg b.w., then after next 4 h ethanol (0.5 and 1 g/kg b.w.) or 4-MP (15 and 50 mg/kg b.w.) was administered by intraperitoneal injection. These solutions were made *ex tempore* in sterilized saline and administered at a constant value of 0.5 cm^3/100 g b.w. of the rat.

Eight hours after the administration of antidote, animals were anaesthetized. Blood was collected to the heparin-Li tube from internal carotid artery, centrifuged at 3000 rpm for 10 min and then plasma was taken for the subsequent analysis. Alanine (ALT) and aspartate (AST) aminotransferase activity and total bilirubin level were measured in auto-analyzer (Liasys, Italy) using commercial kit (Cormay, Poland).

The obtained results were analysed statistically using STATISTICA 5.0. Continuous data were compared in experimental groups using the Kolmogorov-Smirnov test. Statistical significance of differences between control and study groups was analysed by a U Mann-Whitney test. The 0.05 level of probability was used as the criterion for significance.

RESULTS

There were no significant differences in ALT and AST activity and total bilirubin level in rats kept in N_2O/O_2 atmosphere without any other xenobiotics when compared with unexposed control (Fig. 1, 2, 3).



I – N_2O, II– N_2O+ methanol, III– N_2O + methanol + 4-MP 15 mg/kg b.w., IV – N_2O + methanol + 4-MP 50 mg/kg b.w., V – N_2O + methanol + ethanol 0.5 g/kg b.w., VI – N_2O + methanol + ethanol 1.0 g/kg b.w., * $p< 0.05$, ** $p<0.01$, *** $p<0.001$

Fig. 1. Relative alanine aminotransferase activity in rat plasma

Mean ALT activity was increased in rats placed in N_2O/O_2 atmosphere (group I: 61.33 IU/L), but it was not statistically significant *vs.* control (56.12 IU/L). The statistically significant decrease in the mean ALT activity was observed in rats after the administration of methanol (group II: 43.50 IU/L) and methanol with a lower dose of 4-MP (group III: 45.14 IU/L). However, the significant increase in ALT activity was seen only in rats receiving methanol with a higher dose of ethanol (group VI: 69.33 IU/L).

The AST plasma activity was significantly elevated (86.00 IU/L) in rats treated with ethanol as an antidote, in both administered doses (group V and VI: 105.00 and 108.17, respectively) when compared with control.

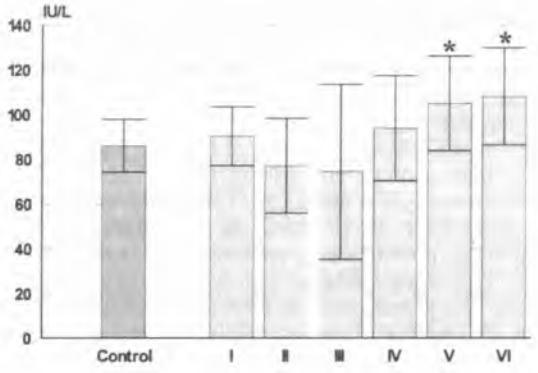


Fig. 2. Relative aspartate aminotransferase activity in rat plasma

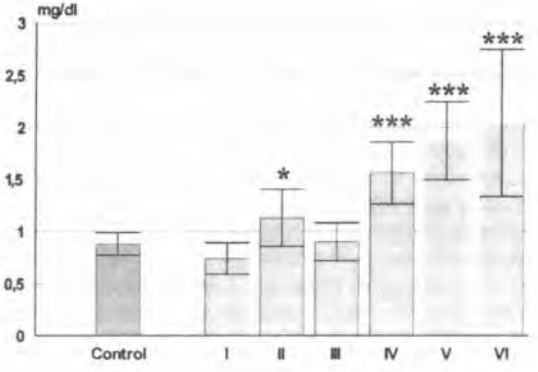


Fig. 3. Relative total bilirubin level in rat plasma

The total bilirubin level was significantly increased in rats treated with methanol (group II; 1.13 mg/dl) when compared with control (0.88 mg/dl). Higher elevation of the bilirubin level was observed in groups exposed to 50 mg of 4-MP and both tested doses of ethanol that were administered after methanol poisoning (groups; IV, V, VI: 1.56; 1.87 and 2.04, respectively).

DISCUSSION

The absence of significant differences in the examined biochemical parameters in rats kept in N_2O/O_2 atmosphere compared with the control, indicates that the gas mixture did not affect the determined parameters. However, in rats intoxicated with methanol (3 g/kg) the activity of ALT was significantly reduced compared with the control. A different effect was reported by Skrzydlewska et al. (10), who observed ALT activity increase after methanol administration at the dose twice higher than that used in our study. This phenomenon may be secondary to the

inhibition of methanol activity or its metabolites on transcriptional level of the enzyme or to the moderate inhibitory effect on its catalytic performance. A similar effect was observed in the methanol intoxicated rats treated with 15 mg 4-MP. It indicates that the administered dose of 4-MP did not affect methanol influence on ALT activity. It should be noted that there were no significant changes when methanol was administered with a higher dose of 4-MP (50 mg/kg) and a lower dose of ethanol (0.5 g/kg). However, a higher dose of ethanol (1 g/kg) in intoxicated rats significantly increased ALT activity. Thus, it seems that there is an adverse interaction between both administered alcohols.

There were no significant changes in AST activity 12 h after methanol (3 g/kg) administration (77.17 IU/L), when compared with the control (86.00 IU/L). Unlike our results, a significant increase in AST activity in rats exposed to methanol (7 g/kg) 2 h before blood examination was reported by Kadiiska (7). In another study, 6 hours after methanol intoxication (6 g/kg) a significant increase of AST activity was found (10). These differences in the obtained results seem to come from various methanol doses. Similarly, there were no significant changes in the rats treated with both tested doses of 4-MP versus control. Contrary to 4-MP, there was a significant increase in AST activity in rats administered with 0.5 g/kg as well as 1 g/kg of ethanol after methanol ingestion. Such interactions could be the consequence of accumulation of toxic metabolites of both alcohols, that results in disturbances in red-ox and acid-basic balance and energetic status and finally may lead to changes in transmembrane permeability and/or hepatocyte injury.

After methanol ingestion, the total bilirubin concentration in plasma was significantly elevated compared to the control. The increase in total bilirubin level progressed when a higher dose of 4-MP and both tested doses of ethanol were administered to the rats treated with methanol. It was shown that ethanol exerted a higher adverse effect than 4-MP in relation to bilirubin level in rat plasma.

The evaluation of ethanol versus 4-MP in methanol poisoning in respect to the impact on ALT and AST activity and total bilirubin level, seems to indicate that 4-MP causes minor adverse effects as antidote in comparison with ethanol.

REFERENCES

1. Burdan F.: Developmental toxicity evaluation of ibuprofen and tolmetin administered in triple daily doses to Wistar CRL: (WI) WUBR rats. Birth Defects Res. Part B. Dev. Reprod. Toxicol., 71, 321, 2004.
2. Chyżyńska M. et al.: Effects of ethanol or 4-methylpyrazol on pH of the blood and renal morphology in rat N_2O/O_2 model after methanol administration. Annales UMCS, Sectio D, 60, 118, 2005.
3. Eells J.T. et al. Development and characterization of a rodent model of methanol-induced retinal and optic nerve toxicity. Neurotoxicology, 21, 321, 2000.
4. Eells J.T. et al.: The regulation of one-carbon oxidation in the rat by nitrous oxide and methionine. Arch. Biochem. Biophys., 219, 316, 1982.
5. Hantson P., Mahieu P.: Pancreatic injury following acute methanol poisoning. J. Toxicol. Clin. Toxicol., 38, 297, 2000.
6. Hantson P. et al.: Ethanol therapy for methanol poisoning: duration and problems. Eur. J. Emerg. Med., 9, 278, 2002.
7. Kadiiska M.B., Mason R.P.: Acute methanol intoxication generates free radicals in rats. An ESR spin trapping investigation. Free Radic. Biol. Med., 28, 1106, 2000.
8. McCoy H.G. et al.: Severe methanol poisoning. Application of a pharmacokinetic model for ethanol therapy and hemodialysis. Am. J. Med., 67, 804, 1979.
9. Megarbane B. et al.: Treatment of acute methanol poisoning with fomepizole. Intensive Care Med., 27, 1370, 2001.
10. Skrzydlewska E., Szynaka B.: Ultrastructural evaluation of hepatocytes membranes. Rocz. Akad. Med. Białymst., 42, suppl. 2, 39, 1997.

SUMMARY

The aim of the study was to evaluate selected biochemical parameters of liver function in rats treated with ethanol or 4-methylpyrazole after methanol poisoning. The study was performed on the rats kept in N_2O/O_2 atmosphere to achieve a close response of rat organism to methanol intoxication as observed in humans. After four-hour exposure to N_2O/O_2 atmosphere rats were intragastrically administered with methanol at a dose of 3 g/kg. Either ethanol (0.5 and 1 g/kg) or 4-methylpyrazole (15 and 50 mg/kg) was administered intraperitoneally four hours later. Alanine and aspartate aminotransferase activity and total bilirubin concentration were measured in blood plasma collected eight hours after the last injection. Statistically significant decrease in ALT activity was observed in rats administered with methanol or methanol with a lower dose of 4-MP compared with the controls. However, a significant increase in ALT activity was observed only in the group receiving methanol together with a higher dose of ethanol. The AST activity was significantly increased in rats treated with ethanol as an antidote, in both used doses when compared with the control. The total bilirubin level was significantly increased in rats treated with methanol versus control. However, a higher elevation of the total bilirubin level was observed in groups exposed to 5 mg of 4-MP and both tested doses of ethanol that were administered after methanol poisoning. In respect to all the examined biochemical factors 4-methylpyrazole caused minor adverse effects in methanol poisoning in rats.

Porównawcza ocena wpływu etanolu i 4-metylopirazolu na aktywność aminotransferazy alaninowej i asparaginianowej oraz na stężenie bilirubiny całkowitej w osoczu krwi szczurów N_2O/O_2 zatrutych metanolem

Celem pracy była ocena wybranych parametrów biochemicznych funkcji wątroby u szczurów zatrutych metanolem, którym następnie podawano etanol lub 4-metylopirazol. Badania przeprowadzono na zwierzętach przebywających w atmosferze N_2O/O_2, co pozwala na uzyskanie podobnej reakcji organizmu na metanol do obserwowanej u ludzi. Zwierzętom przebywającym przez 4 godziny w atmosferze N_2O_2 podawano dożołądkowo metanol (3 g/kg), a następnie po kolejnych 4 godzinach etanol (0,5 i 1,0 g/kg i.p.) lub 4-metylopirazol (15 i 50 mg/kg i.p.). Po 8 godzinach pobierano krew, a następnie w osoczu oznaczono aktywności aminotransferazy alaninowej i asparaginianowej oraz poziom bilirubiny całkowitej. Po podaniu samego metanolu oraz metanolu z niższą dawka 4-MP zaobserwowano istotne obniżenie aktywności ALT w porównaniu z grupą kontrolną. Istotny wzrost aktywności ALT wystąpił jedynie u zwierząt, u których jako odtrutki użyto etanolu w wyższej dawce. Zastosowanie etanolu w dawce 0,5 i 1 g/kg u zwierząt zatrutych metanolem powodowało także istotny wzrost aktywności AST. Stężenie bilirubiny całkowitej było istotnie wyższe w stosunku do kontroli już po podaniu samego metanolu. Dalszy wzrost stężenia bilirubiny całkowitej wystąpił po podaniu 50 mg 4MP i etanolu w dawce 0,5 i 1 g/kg. W odniesieniu do przebadanych parametrów biochemicznych u szczurów zatrutych metanolem 4-metylopirazol wykazywał mniejsze działanie niepożądane w porównaniu z etanolem.