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The plasma activity of α-amylase in methanol intoxicated rats N_2O/O_2 after ethanol or 4-methylpyrazole administration

Ethanol is a worldwide used antidote in methanol poisoning due to its competitive alcohol dehydrogenase inhibitor activity. It has been recently found that the administration of 4-methylpyrazole (4-MP; Fomepizole) is effective and safe in case of these poisons (15). 4-Methylpyrazole is an alcohol dehydrogenase (ADH) inhibitor. The enzyme catalyzes the oxidation of alcohols. Some of the P450 isoenzymes including CYP2E1 are also inhibited by 4-MP (3, 4, 5, 8). Numerous side-effects are observed when ethanol is chosen for methanol intoxication therapy. Central nervous system depression is a major adverse effect, specially, when the doses of ethanol used to saturate alcohol dehydrogenase ADH at therapeutic level are about 0.5–1.0 g/kg. This may lead to respiratory depression in some cases. Pancreatitis or hypoglycaemia could also be partially related either to the methanol poisoning itself or to the ethanol therapy (9, 10, 14). Therefore, it seems that ethanol therapy during methanol poisoning may lead to some interactions between both alcohols.

The absence of an adequate animal model has been a hindrance in estimating the comprehensive adverse effect of methanol in humans under various exposure scenarios (6). Rats and mice do not exhibit the methanol poisoning syndrome observed in primates. However, the folate-reduced rats prepared by the synthetase methionine (E.C.2.1.1.13) inhibition using nitrous oxide (N_2O) decrease in folate levels equivalent to those found in methanol intoxicated humans (7). Therefore, rats kept in N_2O/O_2 mixture (1:1 v/v) atmosphere were used in this study.

No study of the comparison between adverse effects of ethanol and 4-MP in methanol intoxication was found in the literature. This investigation was carried out to evaluate the effect of ethanol and 4-MP on rat N_2O/O_2 α-amylase plasma level during methanol poisoning.

MATERIAL AND METHODS

The experiment was approved by the Local Ethical Committee at the Medical University of Lublin (461/2004). Male Wistar rats (180–220 g) were kept in the N_2O/O_2 atmosphere to reach similar response to methanol intoxication like that observed in humans (6).

Chemicals. All chemicals from commercial sources were of the highest quality and were used without further purification. Ethanol and methanol were obtained from POCh (Gliwice, Poland) and 4-methylpyrazole was purchased from Sigma Chemical Co. (St. Louis, USA). All solutions were prepared immediately prior to their use. The kit for determination of amylase activity was ordered from Cormay (Polska).

Animal study. All rats except control group were placed in a Plexiglass chamber (22 x 55 x 22 cm) and exposed to a mixture of N_2O/O_2 (1:1; flow rate 2 litres/min) for 4 h before the administration of methanol or saline. N_2O/O_2 exposure was continued throughout the experiment.

Methanol was administered *per os* at a dose of 3 g/kg. Then, after next 4 h ethanol (0.5 and 1 g/kg) or 4-MP (15 and 50 mg/kg) was given by i. p. injection. These solutions were made ex tempore in sterilized saline and administered in constant value of 0.5 cm^3/100 g of the rat. Rats were assigned randomly to one of the eleven groups (eight animals per each group). The substances were administered as follows: control – group I (saline); group II – N_2O/O_2; group III – N_2O/O_2, methanol 3 g/kg; group IV – N_2O/O_2, methanol 3 g/kg + 4-MP 15 mg/kg; group V – N_2O/O_2, methanol 3 g/kg + 4-MP 50 mg/kg; group VI – N_2O/O_2, 4-MP 15 mg/kg; group VII – N_2O/O_2, 4-MP 50 mg/kg; group VIII – N_2O/O_2, methanol 3 g/kg + ethanol 0.5 g/kg; group IX – N_2O/O_2, methanol 3 g/kg + ethanol 1 g/kg; group X – N_2O/O_2, ethanol 0.5 g/kg; group XI – N_2O/O_2, ethanol 1 g/kg.

α-Amylase assay. After 16 h from the beginning of the experiment the blood from the cervical artery was collected to the heparinised-(Li) tube to determine α-amylase activity in the rat plasma. The procedure was performed under slight pentobarbital anaesthesia. The blood was centrifuged at 3,000 x g for 5 min at 4°C and the plasma was frozen at –75°C until assayed. α-Amylase activity in the rat plasma was measured spectrophotometrically at λ = 405 nm using microreader PwerWave, BIO-TEK. The reading was repeating after exactly 1, 2 and 3 minutes against water. The assay was carried out according to the details described in the kit procedure.

Statistical analysis. The obtained date of α-amylase activity was analysed statistically using Statistica 5.0 program. Statistical significance of differences between control and study groups was analysed by the U Mann-Whitney test. In all cases, the minimum level of significance was taken as $p<0.05$.

RESULTS

The mean value of α-amylase activity in plasma in rats placed in N_2O/O_2 atmosphere was not statistically significant versus control group (Tab. 1). Therefore, subsequently comparisons were performed between the study group and control. In rats receiving 3 g/kg of methanol α-amylase activity in plasma was not significantly changed versus control. No significant decrease in the mean value of α-amylase activity in rat plasma was also observed after administration of 4-methylpyrazole (15 mg/kg and 50 mg/kg) and ethanol of (0.5 g/kg and 1 g/kg). However, the statistical significance was observed when 1 g/kg of ethanol was administered to rats ingested with methanol.

Table 1. Rat plasma α-amylase activity (UI/L). Results are mean (M)±SD for seven observations in every group versus control. Numbers of groups are described in material and methods section

No. of groups	Min	Max	M	SD	Me	p
I (control)	1073.00	1510.00	1272.14	139.916	1262.00	
II	986.00	1465.00	1249.14	172.570	1243.00	0.8480
III	1051.00	1445.00	1212.57	153.352	1144.00	0.4822
IV	929.00	1461.00	1185.29	224.738	1092.00	0.5653
V	756.00	1884.00	1255.57	442.619	1287.00	0.8480
VI	889.00	1543.00	1221.43	220.692	1243.00	0.7494
VII	1005.00	1591.00	1249.00	196.887	1243.00	0.7494
VIII	951.00	1310.00	1191.00	121.900	1233.00	0.2774
IX	1215.00	1960.00	1614.57	271.650	1526.00	0.0181
X	989.00	1422.00	1226.86	175.393	1299.00	0.7494
XI	880.00	1422.00	1192.29	226.716	1299.00	0.7494

DISCUSSION

It is well known that the liver plays a key role in metabolising of alcohol. About 80% of ingested alcohol is oxidised in this way, while the other alcohol amount is metabolised by peripheral tissue. In rats a catalase-peroxidase system is considered to be responsible for oxidising methanol to formaldehyde. Then, formaldehyde is oxidised by formaldehyde dehydrogenase (FDH), an enzyme that requires reduced glutathione as a cofactor (12). Moreover, other dehydrogenases also play a role in oxidation of formaldehyde. As a result, the increase in the NADH/NAD^+ ratio is observed (13). This leads to a rise in xantine oxidase activity which is the principal source of superoxide anion (11). Recently, it has been showed that metabolism of ethanol and methanol is accompanied by reactive oxygen species production (1, 2).

In rats subjected to a higher dose of ethanol which previously had received methanol, the highest activity of α-amylase was found ($p<0.5$). It may be the consequence of free radical production that may result in lipid peroxidation and structural protein failure. It brings about cell membrane permeability for some enzymes like α-amylase and therefore, the increase of the enzyme activity in plasma could be observed. It suggests that there is the disadvantage interaction between methanol and ethanol administered at a dose 1 g/kg. The ethanol in doses 0.5 g/kg to 1 g/kg is used in humans intoxicated with methanol to obtain effective, therapeutic effect of alcohol dehydrogenase inhibition.

CONCLUSION

The lack of changes in plasma α-amylase activity after methanol and ethanol administered alone versus control group and a significant increase of α-amylase activity in rats after methanol (3 g/kg) and subsequent ethanol (1 g/kg) administration suggests an interaction taking place between both alcohols.

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SUMMARY

In the study the potential interaction of methanol/ethanol and methanol-4-methylpyrazole within the scope of the α-amylase concentration in rat plasma was investigated. The experiment was carried out on rats exposed to N_2O/O_2 mixture (1:1 v/v) which allow similar response of rat organism to methanol poisoning to that observed in human. The rats were exposed to N_2O/O_2 mixture 4h before the administration of methanol or saline. N_2O/O_2 exposure continued throughout the experiment. Methanol was administered *per os* at a dose 3 g/kg, then, after next 4 h ethanol (0.5 g/kg or 1 g/kg) or 4-methylpyrazole (15 mg/kg or 50 mg/kg) was administered by i. p. injection. α-Amylase in rat plasma was essayed spectrophotometrically at $\lambda = 405$ nm. The mean value of α-amylase activity in plasma in rats placed in N_2O/O_2 atmosphere was not statistically significant versus control group. In rats receiving 3 g/kg of methanol the α-amylase activity in plasma was not significantly changed versus control. No significant decrease in the mean value of α-amylase activity in rat plasma was also observed after administration of 4-methylpyrazole (15 mg/kg and 50 mg/kg) and ethanol of (0.5 g/kg and 1 g/kg). However, the statistical significance increase was observed when 1 g/kg of ethanol was administered to rats ingested with 3 g/kg of methanol. The lack of changes in plasma amylase activity after methanol and ethanol administered alone versus control group and the significant increase of amylase in rats after methanol (3 g/kg), and subsequent ethanol (1 g/kg) administration suggests an interaction between both alcohols.

Aktywność α-amylazy w surowicy krwi szczurówN_2O/O_2 zatruwanych metanolem, a następnie poddanych działaniu etanolu lub 4-metylopirazolu

Celem badań była ocena potencjalnych interakcji metanolu z etanolem oraz metanolu z 4--metylopirazolem w odniesieniu do aktywności α-amylazy w surowicy krwi szczurów. Doświadczenie zostało przeprowadzone na szczurach poddanych ekspozycji na mieszaninę N_2O/O_2 (1:1 v/v), co pozwala uzyskać podobną reakcję organizmu szczura na metanol do tej obserwowanej u ludzi. Szczury były narażone na mieszaninę N_2O/O_2 na 4 godziny przed podaniem metanolu oraz przez cały okres trwania doświadczenia. Metanol był podawany sondą dożołądkową w dawce 3 g/kg, następnie po 4 godzinach podawano i. p. etanol (0,5/kg lub 1 g/kg) bądź 4-metylopirazol (15 mg/kg lub 50 mg/kg). α-Amylaza była oznaczana spektrofotometrycznie przy długości fali 405 nm. Średnia aktywność α-amylazy w

osoczu krwi u szczurów przebywających w atmosferze N_2O/O_2 nie różniła się w sposób istotny w stosunku do grupy kontrolnej. Również u zwierząt, którym podawano sam metanol (3 g/kg), sam etanol (0,5/kg lub 1 g/kg) oraz sam 4-metylopirazol (15 mg/kg lub 50 mg/kg) nie zaobserwowano istotnych zmian aktywności α-amylazy w osoczu krwi. Jednak u szczurów, które po podaniu metanolu otrzymały etanol w dawce 1 g/kg, doszło do istotnego wzrostu aktywności α-amylazy w surowicy krwi. Wzrost aktywności α-amylazy u szczurów otrzymujących zarówno metanol (3 g/kg), jak i etanol (1 g/kg), przy braku zmiany po podaniu obydwu alkoholi pojedynczo, sugeruje interakcję pomiędzy metanolem i etanolem w odniesieniu do aktywności α-amylazy w osoczu krwi.