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Effects of ethanol or 4-methylpyrazol on pH of the blood and renal morphology in rat N_2O/O_2 model after methanol administration

The main therapy schedule in methanol poisoning is administration of alcohol dehydrogenase (ADH) inhibitors to prevent formate accumulation. Earlier study showed that ADH inhibitor – 4-methylpyrazol (4-MP) may inhibit catalase (1) and microsomal alcohol oxidizing system activity (2, 3) and activated human skeletal muscle lactate dehydrogenase activity (4). Ethanol has been used for the treatment in methanol poisoning for over 50 years because it shows more than twenty--fold affinity to the ADH than methanol. In 2000 the FDA (USA Food and Drug Administration) approved the 4-methylpyrazol for the treatment of methanol poisoning (5).

No study of the comparison between side-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 kidney and blood pH equilibrium during methanol poisoning.

Both methanol and ethanol cause changes in electrolytes concentration in the organism but metabolites of 4-MP are excreted by kidney. Therefore, it may be supposed that there are the reciprocal interactions between methanol and administered antidote.

The aim of the study was to evaluate side-effects caused by ethanol and 4-MP during methanol intoxication as a result of interactions between methanol/ethanol and methanol/4-MP.

MATERIAL AND METHODS

The experiment was performed in accordance with the Guide for the Care and Use of Laboratory Animals, DHEW Publication No(NIH) 85-23, 1985 and approved by the Local Ethical Committee at the Medical University of Lublin. Male Wistar rats (180–220 g) were kept in the N_2O/O_2 atmosphere to reach a maximum close response of organism to methanol intoxication like that observed in humans (6).

All rats except 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 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 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 ip. injection. These solutions were made *ex tempore* in sterilized saline and administered in constant value of 0.5 cm^3/100 g b.w. 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 b.w.; group IV – N_2O/O_2, methanol 3 g/kg b.w. + 4-MP 15 mg/kg b.w.; group V – N_2O/O_2, methanol 3 g/kg b.w. + 4-MP 50 mg/kg b.w.; group VI - N_2O/O_2, 4-MP 15 mg/kg b.w.; group VII – N_2O/O_2, 4-MP 50 mg/kg b.w.; group VIII – N_2O/O_2, methanol 3 g/kg b.w. + ethanol 0.5 g/kg b.w.; group IX – N_2O/O_2, methanol 3 g/kg b.w. + ethanol 1 g/kg b.w.; group X – N_2O/O_2, ethanol 0.5 g/kg b.w.; group XI –N_2O/O_2, ethanol 1 g/kg b.w.

After 16 h from the beginning of the experiment the blood from the cervical artery under slight pentobarbital anaesthesia was collected to the heparinised capillary tube to determine gasometric parameters (pH) using Ciba Corning 248 apparatus. After decapitation, the kidneys were removed and fixed in 10% buffered –formaline (pH 7.2) for 24 h. Then, the tissue samples were processed routinely, embedded in paraffin block, sectioned and stained with hematoxylin and eosin (H & E), periodic acid Schiff (PAS), PAS after prior diastase digestion, van Gisons and Gomori's silver impregnation. The slides were evaluated on light microscope (Olympus BX45).

Statistical analysis. The obtained results of pH value were analysed statistically using STATISTICA 5.0. program. Statistical significance of differences between control and study groups was analysed by U Mann-Whitney test. In all cases, the minimum level of significance was taken as $p<0.05$.

RESULTS

The results of blood pH value are shown in Table 1. Mean value of pH was decreased in rats placed in N_2O/O_2 atmosphere (group II), but it was not statistically significant vs. control. Significant statistical decrease in mean pH value was observed in rats after administration of methanol (group III) and ethanol (1 g/kg b.w., group XI) alone as well as in group which received both methanol and ethanol (1 g/kg b.w., group IX). In rats, which received: methanol + 15 mg 4-MP (group IV); methanol + 50 mg 4-MP (group V) and methanol + 0.5 g ethanol (group X) statistical significant difference vs. control was not observed.

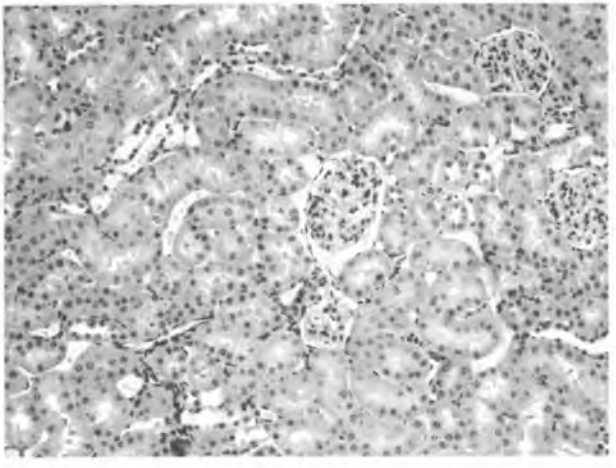

Fig. 1. Histological appearance of the kidney in rat from the control group (H & E, magn. x200)

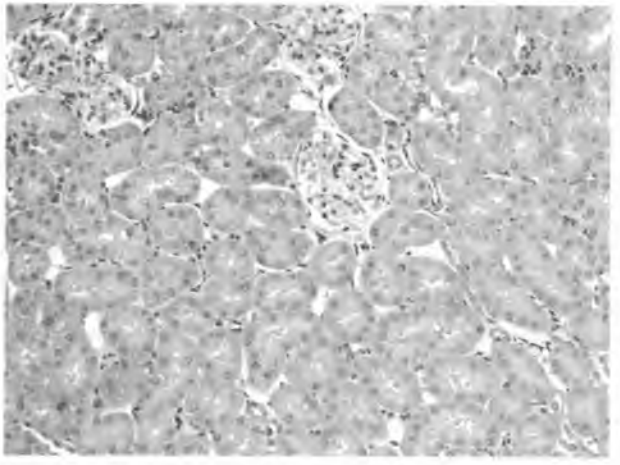

Fig. 2. Histological appearance of the kidney in rat from the group after administration of 3 g methanol/kg b.w. (H & E, magn. x100)

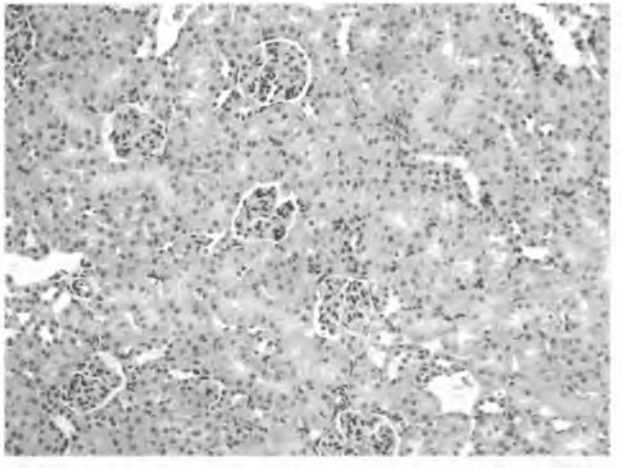

Fig. 3. Histological appearance of the kidney in rat from the group after administration of 3 g methanol/kg b.w. and 50 mg 4-MP/kg b.w. (H & E, magn. x100)

The microscopic appearance of the rat from the experimental was similar to the control (Fig. 1–4). Only mild hyperaemia both in renal cortex and medulla was observed in some of the experimental groups, i.e. 50 mg 4-MP/kg b.w (8/8 rats); 1g ethanol (8/8 rats), methanol +1 g ethanol (7/8 rats), methanol (6/8rats) and methanol and 50 mg 4-MP (6/8 rats).

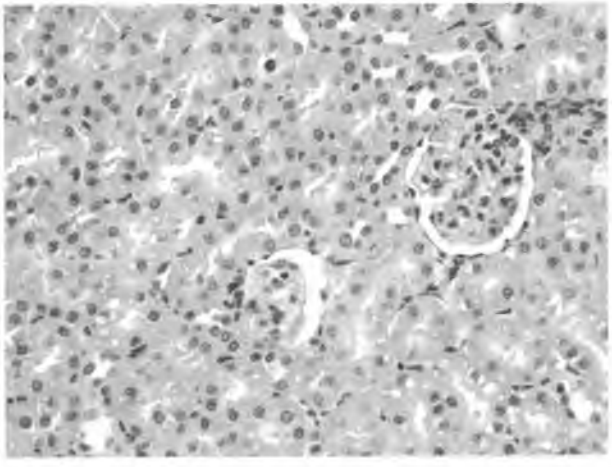

Fig. 4. Histological appearance of the kidney in rat from the group after administration of 3g methanol/kg b.w. and 1 g ethanol/kg b.w. (H & E, magn. x200)

DISCUSSION

On the basis of the values presented in Table 1 a significance decrease in the blood pH was observed after the administration of 3 g methanol, 1 g ethanol and both alcohols in the above mentioned doses. The mechanism of acidosis during methanol or ethanol poisoning is very similar. It is associated with analogous metabolism of both alcohols resulting from the formic or acetic acid formation. Formic acid is stronger than acetic acid, so the decrease in the pH value should be greater in methanol poisoning, than after the ingestion of the same molar equivalent value of ethanol.

Table 1. Blood pH value

No. Groups	N of rats	Minimum	Maximum	Mean	Median	S.D.	P vs. control
I (control)	8	7.303	7.430	7.381	7.381	0.043	
II	6	7.230	7.402	7.334	7.353	0.078	0.5212
III	8	7.231	7.328	7.288	7.291	0.034	0.0066
IV	8	7.347	7.450	7.400	7.401	0.032	0.4381
V	8	7.368	7.425	7.392	7.385	0.022	0.8973
VI	8	7.346	7.451	7.381	7.372	0.034	0.5186
VII	6	7.323	7.381	7.341	7.335	0.022	0.1093
VIII	8	7.320	7.400	7.352	7.342	0.029	0.1967
IX	8	7.271	7.373	7.308	7.300	0.037	0.0097
X	8	7.289	7.438	7.346	7.340	0.051	0.1967
XI	8	7.260	7.362	7.305	7.304	0.037	0.0097

Additionally, in severe methanol intoxication the increase of the $NADH/NAD^+$ ratio is observed, as a result of metabolic transformation. Then, increased transformation of pyruvate to

lactate catalysed by lactic dehydrogenase (LDH) is found. Therefore, the previously existing acidosis is intensified by lactic acidosis.

In our study, after methanol administration the decrease in pH value to 7.288 (control = 7.381) was observed. Higher ethanol doses (1 g/kg b.w.) caused the decrease in pH to 7.305 and after the administration of methanol and ethanol (1 g/kg b.w.) the pH value was 7.308. In the remaining groups there were no significant differences in pH value.

It may be concluded that the decrease in pH value was not intensified when ethanol was given after methanol administration. 4-Methylpyrazol used at doses 15 and 50 mg//kg b.w. did not affect pH level vs. control (pH: 7.381 and 7.341 respectively). Moreover, 4-MP given to rats that were previously administered methanol, normalised pH value.

Despite some changes in pH value, the morphological appearance of kidneys taken from the rats of control and most experimental groups was normal. Mild hyperaemia both in cortex and medulla was observed in rats after the administration of methanol, 50 mg/kg 4-MP and ethanol 1 g/kg b.w. alone as well as methanol with 50 mg/kg 4-MP or ethanol 1 g/kg b.w.

The changes in blood pH after the administration of methanol (group III), 1 g ethanol (group XI), and methanol + 1 g ethanol (group VIII), may suggest the dysfunction of distal renal tubule without changes in the morphological structure of the kidney.

CONCLUSIONS

1. The 4-methylpyrazol normalised pH value in methanol poisoning at both used doses.
2. There were no significant morphological renal changes in the investigated rats compared with the control group.

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SUMMARY

Administration of alcohol dehydrogenase inhibitors (e.g., ethanol, 4-methylpyrazol) in methanol poisoning to prevent formate accumulation is the main therapy schedule. The present study was carried out to evaluate the effect of ethanol (0.5 and 1.0 g/kg) and 4-methylpyrazol (15.0 and 50.0 mg/kg) on the renal morphology and blood pH equilibrium during the methanol poisoning. Male Wistar rats were kept in the N_2O/O_2 atmosphere and dosed with 3 g/kg of methanol. Statistical significant decreases in pH value were found in groups exposed to methanol, higher doses of ethanol and rats dosed with both methanol and ethanol (1 g/kg) when compared with untreated

control group. The 4-methylpyrazol normalized pH value in methanol poisoning. No treatment-related morphological renal changes were observed.

Wpływ etanolu i 4-metylopirazolu na pH krwi i budowę nerki u szczurów w modelu N_2O/O_2 po podaniu metanolu

Celem pracy było porównanie dwóch środków stosowanych jako antidotum w zatruciach metanolem – 4-metylopirazolu oraz etanolu. Badania przeprowadzono na szczurach przebywających w atmosferze N_2O/O_2, co zapewniało reakcję organizmu zwierzęcia podobną do tej, jaką obserwuje się u ludzi w zatruciu metanolem. Zwierzętom podawano sam metanol (3 g/kg p.o.) oraz 4-metylopirazol (15 i 50 mg/kg i.p.) i etanol (0,5 i 1,0 g/kg i.p. Oceniono morfologię nerki szczura w mikroskopie świetlnym oraz oznaczono wartość pH we krwi. Stwierdzono, że 4-metylopirazol normalizował pH krwi szczura zatrutego wcześniej metanolem w obydwu zastosowanych dawkach. Nie zaobserwowano istotnych zmian w obrazie morfologicznym nerki w żadnej z badanych grup zwierząt.