Acta Physiologica Hungarica, Volume 91 (3–4), pp. 235–241 (2004)

Effect of selected alcohol dehydrogenase inhibitors on the human heart lactate dehydrogenase activity – an *in vitro* study

J **Dudka**[1], F **Burdan**[2], Barbara **Madej**[3], Justyna **Szumilo**[1], Edyta **Tokarska**[4], Agnieszka **Korobowicz**[2], R **Klepacz**[1], Monika **Chyżyńska**[1], Elżbieta **Korobowicz**[1]

[1]Department of Clinical Pathomorphology,
[2]Experimental Teratology Unit of the Human Anatomy Department,
[3]Department of Human Anatomy,
[4]Department of Jaw Orthopaedics, Medical University, Lublin, Poland

Received: February 19, 2004
Accepted: June 1, 2004

Metabolic acidosis complicates methanol, ethylene glycol and other alcohol intoxications. It is caused firstly by acid metabolites and secondly by the lactate elevation. The aim of the study was to evaluate the effect of alcohol dehydrogenase (ADH; EC 1.1.1.1) inhibitors and substrates: 4-methylpyrazole (4-MP), cimetidine, EDTA, ethanol and methanol on lactate dehydrogenase (LDH; EC 1.1.1.27) activity. The activity of LDH was determined spectrophotometrically in *in vitro* human heart homogenates with the mentioned compounds at 0.01, 0.1, 1.0 mM concentrations of 4-MP, cimetidine, EDTA, and 12.5, 25.0, 50.0 mM of ethanol and methanol. The LDH activity was significantly inhibited by 0.1 mM ($p<0.05$) and 1.0 mM ($p<0.01$) 4-MP and 1.00 mM EDTA ($p<0.05$). Higher LDH activity vs. control was observed in the samples incubated with all studied ethanol and methanol concentrations but these differences were not statistically significant. Thus, 4-MP was found to be the most effective inhibitor of LDH of all compounds tested. Therefore, such effect of 4-MP seems to be an additional advantage in methanol and ethylene glycol intoxications.

Keywords: cimetidine, EDTA, methanol, 4-methylpyrazole, lactate dehydrogenase, alcohol dehydrogenase inhibitors, human heart, ethanol, LDH

Methanol and ethylene glycol poisoning share many clinical and biochemical characteristics. Both alcohols are metabolised first via alcohol and than by aldehyde

Correspondence should be addressed to
Jarosław Dudka, PhD
Department of Clinical Pathomorphology
Medical University of Lublin
8 Jaczewskiego Str.
PL-20950 Lublin, Poland
Phone: +48-81 742 58 94
Fax: +48-81 740 58 54
E-mail: ave123@wp.pl

dehydrogenases to their toxic acidic metabolites (20). Simultaneously, the $NADH_2$/NAD ratio is significantly elevated (3, 21, 25). Higher $NADH_2$ synthesis leads to a significant reduction in pyruvate by lactate dehydrogenase (LDH). Although LDH can reduce pyruvate as well as oxidize lactate, the preferred pathway is the transformation of pyruvate to lactate. The *K* (equilibrium constant) value for this reaction calculated from the formula $\Delta E'_0 = (0.059/n)\log K$, where $\Delta E'_0$ means potential redox difference and n is the number of transferred electrons, equals 20.000 (22). It means that practically the whole pyruvate is reduced to lactate which results in an increase in lactate in a reaction medium. At late stage of methanol intoxication lactate might also accumulate, mainly due to the respiratory chain inhibition by formate. Those mechanisms at late stages of acute poisonings are responsible for the increase in lactate concentration (1, 2, 10, 16, 24, 29, 30). Hence, acidosis can get worse when lactate is produced by LDH.

Additionally, the dynamics of lactic acid formation depends on the participation of different LDH isoenzymes in a particular tissue. As mentioned above LDH can catalyse pyruvate to lactate and lactate to pyruvate. The preferable direction in each tissue, apart from $NADH_2$/NAD and pyruvate/lactate ratio, depends on the presence of different amounts of isoenzymes. LDH-5 consists of 4M (muscle) subunit and preferably catalyses pyruvate to lactate. LDH-5 in human liver and muscle is present in 94% and 76% of total LDH isoenzymes, respectively. LDH-1 consists of 4H (heart) subunits and is found in plasma and human heart in 60% and preferably oxidises lactate to pyruvate if cell glucose deficit appears. The direction of the transformation of lactate and pyruvate catalysed by the rest of isoenzymes: LDH-2, LDH-3, LDH-4 depends on the quantitative participation of M and H subunit in each tetramer of LDH (7, 8, 9, 31).

Therefore, it seems important to examine the LDH activity with 4-methylpyrazole (4-MP) and ethanol, which are used nowadays as antidotes in methanol and ethylene glycol poisoning. Cimetidine and EDTA, medicines which have a documented inhibitory effect on first stage methanol and ethylene glycol metabolism in humans (11–13) were also used.

Materials and Methods

This experiment was designed in accordance with the international bioethical guidelines and the national law.

Reagents

All chemicals from commercial sources were of the highest quality and were used without further purification. Ethanol was obtained from Polskie Odczynniki Chemicze; (Poland); 4-methylpyrazole and ethylenediaminetetraacetic acid disodium salt (EDTA) were purchased from Sigma Chemical Co. (St. Louis, MO – USA). Cefarm (Poland) supplied cimetidine. LDH-kit was obtained from Cormay (Poland). All solutions were prepared immediately prior to use.

Tissue preparation

Fifteen samples of human heart were taken from human male, aged from 26 to 61 years (median 38.5), 24 to 30 hours after death. The causes of death were traffic accidents. The heart samples were homogenised in sodium phosphate buffer pH 7.5 (ratio 1:4 weight: volume) at room temperature. Final homogenates were centrifuged at 18.000 g for 30 min. The supernatants were diluted 100 times with 0.9% NaCl and immediately used for enzyme assays.

Enzyme assay

All the assays of LDH activity were performed spectrophotometrically by the examination of the rate of NADH depletion at 340 nm using the Express Plus spectrophotometer (Bayer, Germany) with Liquick Cor-LDH 30 kit (Cormay, Poland). The method followed the optimized kinetic method of Deutsche Für Klinische Chemie (26), which is based on the shift of pyruvate to lactate. The reaction mixture contained 50 mM phosphate buffer (pH 7.5), 0.6 mM pyruvate and the examined compound solution. The concentrations 0.01; 0.10; 1.10 mM of cimetidine, EDTA and 4-methyl-pyrazole and 12.5, 25.0 and 50.0 mM ethanol as well as methanol were used in the environment of reactions. Ethanol was applied in such concentration because 50 mM of ethanol is a minimum concentration, which is effective as antidote in methanol poisoning treatment (17, 21). Subsequently the diluted supernatant containing the enzyme was added. After 5 min of incubation at 37 °C, the reaction was triggered by adding 0.25 mM NADH. Enzyme activity was represented as U/l of diluted supernatants.

These results were compared with values obtained under identical conditions where 0.9% of NaCl instead of the compounds examined was used.

Statistical analysis

Data obtained were analyzed using STATISTICA 5.0. The homogeneity was examined using the Shapiro-Wilks test. Statistical significances among groups were calculated using Student's *t*-test. An $\alpha = 0.05$ ($p<0.05$) was considered significant (19).

Results

Eleven from fifteen samples were included in the study. Four samples were excluded because of extreme activities of LDH.

The influence of cimetidine, 4-MP, EDTA, ethanol and methanol on the activity of LDH is presented in Table I and Figure 1. The LDH activity was significantly inhibited with 0.10 and 1.00 mM of 4-MP by 15.37% ($p<0.05$) and 18.43% ($p<0.01$), respectively.

Table I

Lactate dehydrogenase activity (IU/l) in diluted supernatant of human heart homogenate with cimetidine, EDTA, 4-methylpyrazole (4-MP), ethanol and methanol

	Concentration (mM)	M ± SD	Min	Max	Median	Δ%	p
Control		352.64 ± 51.60	274	432	368		
Cimetidine	0.01	331.54 ± 59.29	251	427	346	–5.98	0.384
	0.1	330.82 ± 61.24	246	457	345	–6.19	0.376
	1.00	354.64 ± 58.67	251	458	354	+0.57	0.933
EDTA	0.01	346.27 ± 48.42	263	412	342	–1.81	0.768
	0.1	331.64 ± 49.83	253	398	342	–5.95	0.343
	1.00	296.63 ± 48.98	221	368	315	–15.88	0.017*
4-MP	0.01	321.45 ± 55.50	226	389	336	–8.84	0.187
	0.1	298.45 ± 52.63	216	367	315	–15.37	0.024*
	1.00	287.63 ± 52.39	205	346	307	–18.43	0.008**
Ethanol	12.50	377.91 ± 50.72	307	457	387	+7.16	0.260
	25.00	376.36 ± 58.78	295	498	382	+6.73	0.326
	50.00	384.27 ± 57.63	308	477	374	+8.97	0.190
Methanol	12.50	359.36 ± 47.43	304	438	367	+1.90	0.753
	25.00	360.09 ± 47.19	296	427	373	+2.11	0.727
	50.00	362.73 ± 60.37	246	464	357	+2.81	0.678

Results are mean (M) ± SD for eleven observations
$\Delta\ \% = (M_{sample} \times 100\%/M_{control})–100$
*: $p< 0.05$; **: $p<0.01$ vs control

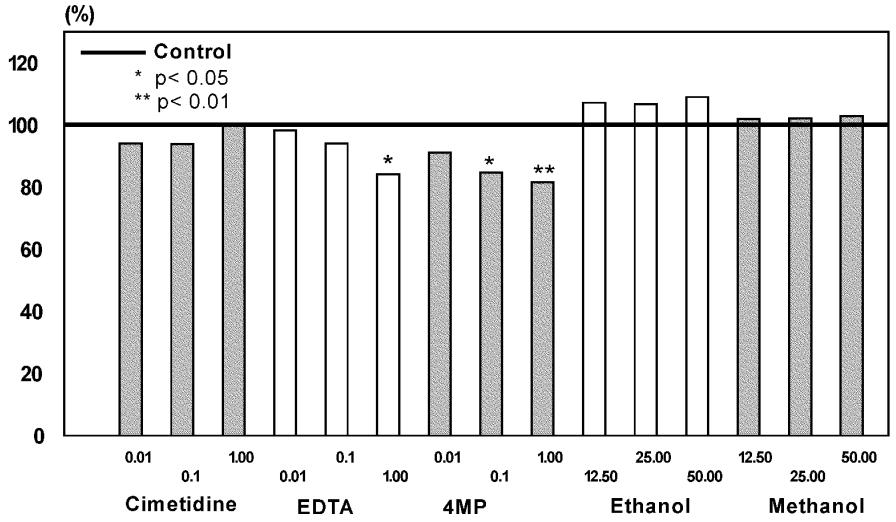

Fig. 1. Human heart homogenate LDH activity as the percentage of control

A similar effect (inhibition by 15.88%) on LDH activity with 1.00 mM of EDTA in the environment of reaction was observed. Only a slight and insignificant inhibitory effect of LDH was found at lower concentrations of EDTA. In our study both methanol and ethanol showed statistically insignificant suppression of LDH activity at all concentrations tested.

Discussion

The most serious problem in methanol and ethylene glycol poisonings is metabolic acidosis resulting from acid metabolites of these alcohols. A rise in $NADH_2$/NAD ratio in alcohol and aldehyde metabolism stimulates LDH activity to produce lactate. Elevated concentration of lactate is added to the existing acidosis produced by formate or glioxylic acid. It would be interesting to see if alcohol dehydrogenase inhibitors can also inhibit LDH in human tissue. In our previous study (14, 15) the effect of 4-MP, cimetidine, EDTA, methanol and ethanol on human serum and skeletal muscle lactate dehydrogenase activity was examined. It was found that cimetidine, EDTA and 4-MP could change LDH activity. 4-MP and cimetidine inhibit human serum LDH, but lead to the rise in LDH activity in human skeletal muscle homogenates. It seems that such differences may depend on the pattern of LDH isoenzymes in the particular tissue.

The LDH enzyme is a tetramer composed of monomeric subunits encoded by two single copy genes, LDH-A and LDH-B (18). LDH-A (LDH-5) isoenzyme converts pyruvate to lactate in anaerobic conditions. LDH-5 has been in the main described in liver (7), skeletal muscle (8) and astrocytes (5). The activity of LDH-5 is lower in other cells, e.g. neurons, erythrocytes and cardiomyocytes. LDH-1 consists of 4H (heart) subunits and is present in plasma and human heart in 60% and it preferentially oxidises lactate to pyruvate. Therefore, the effect of some drugs may depend on the particular tissue. From toxicological point of view it should be given if the substances tested are able or not to inhibit lactate synthesis in the particular tissue, and it also should be given which isoenzyme is inhibited. In that context the power of the inhibition is not so important. This study presents changes of the total LDH activity in human heart with known inhibitors of ADH (11, 12, 13).

4-Methylpyrazole (4-MP) which is a strong and relatively safe ADH inhibitor, is widely used for the treatment of alcohol poisoning, especially in patients who refuse dialysis or cannot be treated with ethanol (4, 6, 32). Recent studies have revealed that total LDH (LDH-1-LDH-5) was inhibited with 0.10 and 1.00 mM 4-MP by 15.37% and 18.43%, respectively. Furthermore, similar effect has been recently observed when 4-MP was used to inhibit LDH activity in human serum (14). Sarkola et al. (28) also observed significant correlation between the effect of 4-MP and the alcohol-induced lactate elevation in human plasma. However, in human liver and skeletal muscle the LDH activity was elevated with 4-MP (15). Therefore, such differences of drug response to LDH may result from the different pattern of LDH isoenzymes. As mentioned above, LDH-5 is the main isoenzyme in liver (94%) and human skeletal

muscle (76%) and it constitutes only small fraction in serum. In serum however, the predominant isoenzyme is rather LDH-2, than LDH-1 and only about 10% is the sum of LDH-3, LDH-4 and LDH-5. The types of LDH-1 and LDH-5 are different in their physical characteristics such as binding constants of substrates and catalytic sites (8). This may explain why the reaction with the same compounds may occur in a different way in different tissues.

In our study, there were significant changes in LDH activity with the highest (1.00 mM) tested concentration of EDTA. In that case the LDH activity decreased by 15.88% ($p<0.05$) vs. control. In earlier study it was found that LDH and ADH structures incorporate Zn^{+2} which is believed to play a key role in catalysis (23). The functional role of the metal is demonstrated by the inhibition of enzyme activity by metal binding agents (13, 22, 26). Moreover, as it was previously demonstrated (13), EDTA inhibited the ADH activity in human liver. In this regard current research included EDTA as an effective antidote in heavy metals investigation.

It has been observed that the activity of LDH was in the samples incubated with ethanol and methanol at all the concentrations like in the controls. However, these differences were not statistically significant. In our previous study (14) insignificant statistical changes in the LDH activity in serum with ethanol was also found.

Our study reveals that 4-MP is found to be the most effective inhibitor of the human heart LDH of all the compounds tested. Therefore, such effect of 4-MP seems to be the additional advantage in methanol and ethylene glycol intoxications.

REFERENCES

1. Aabakken L, Johansen KS, Rydningen EB et al.: Osmolal and anion gaps in patients admitted to an emergency medical department. Hum. Exp. Toxicol. 13, 131–134 (1994)
2. Ammar KA, Heckerling PS: Ethylene glycol poisoning with a normal anion gape caused by concurrent ethanol ingestion: importance of the osmolal gap. Am. J. Kidney Dis. 27, 130–133 (1996)
3. Baud FJ, Borron SW, Bavoux E et al.: Relation between plasma lactate and blood cyanide concentrations in acute cyanide poisoning. Br. Med. J. 312, 26–27 (1996)
4. Bekka R, Borron SW, Astier A et al.: Treatment of methanol and isopropanol poisoning with intravenous fomepizole. J. Toxicol. Clin. Toxicol. 39, 59–67 (2001)
5. Bittar PhG, Charnay Y, Pellerin L et al.: Selective distribution of lactate dehydrogenase isoenzymes in neurons and astrocytes of human brain. J. Cereb. Blood Flow Metab. 16, 1079–1089 (1996)
6. Brent J, McMartin KE, Philips S et al.: Fomepizole for the treatment of methanol poisoning. N. Engl. J. Med. 344, 424–429 (2001)
7. Brooks GA, Dubouchaud H, Brown M et al.: Role of mitochondrial lactate dehydrogenase and lactate oxidation in the intracellular lactate shuttle. Proc. Natl. Acad. Sci. USA. 96, 1129–1134 (1999)
8. Buono RJ, Sheffield JB: Changes in expression and distribution of lactate dehydrogenase isoenzymes in the developing chick retina. Exp. Eye Res. 53, 199–204 (1991)
9. Buono RJ, Lang RK: Hypoxic repression of lactate dehydrogenase-B in retina. Exp. Eye Res. 69, 685–693 (1999)
10. Cytryn E, Futeral B: Lactate accumulation in methanol poisoning. Lancet. 2, 56 (1983)
11. Dawidek-Pietryka K, Dudka J: Effect of some ADH inhibitors on microsomal alcohol oxidizing system activity in vitro. Roczn. Panstw. Zakl. Hig. 52, 337–345 (2001)

12. Dawidek-Pietryka K, Dudka J, Szczepaniak S: Inhibition alcohol dehydrogenase by selected compounds in methanol end ethylene glycol transformation. Bromat. Chem. Toksykol. 33, 155–160 (2000)
13. Dawidek-Pietryka K, Szczepaniak S, Dudka J, Mazur M: In vitro studies of human alcohol dehydrogenase inhibition in the process of methanol and ethylene glycol oxidation. Arch. Toxicol. 72, 604–607 (1998)
14. Dudka J, Dawidek-Pietryka K, Burdan F, Madej B: Lactate dehydrogenase activity changes in the presence of some alcohol dehydrogenase inhibitors. Progress in Medical Research, http//www.progmedres.com/pdf/vol2/3667, pdf (2004)
15. Dudka J, Burdan F, Korobowicz A, Klepacz R, Korobowicz E: Human skeletal muscle lactae dehydrogenase activity in the presence of some alcohol dehydrogenase inhibitors. Basic Clin. Pharmacol. Toxicol. 95, 38–42 (2004)
16. Eder AF, McGrath CM, Dowdy YG et al.: Ethylene glycol poisoning: toxicokinetic and analytical factors affecting laboratory diagnosis. Clin. Chem. 44, 168–177 (1998)
17. Ellenhorn MJ, Barceloux DG (1988): Medical Toxicology. Diagnosis and treatment of human poisoning. Elsevier, New York.
18. Evers J, Kaplan NO (1973): Advances in Enzymology. Lactate dehydrogenase: structure and function, Eds Meister A, Wiley Hoboken.
19. Gad SC (2001): Statistics for toxicologist. In: Principles and Methods of Toxicology, eds Taylor & Francis, Philadelphia, pp. 285–364.
20. Jacobsen D, McMartin KE: Antidotes for methanol and ethylene glycol poisoning. Clin. Toxicol. 35, 127–143 (1997)
21. Jacobsen D, McMartin KE: Methanol and ethylene glycol poisonings. Mechanism of toxicity, clinical course, diagnostic and treatment. Med. Toxicol. 1, 309–334 (1986)
22. Kafarski P, Lejczak B (1994): Bioorganic Chemistry. PWN, Warszawa.
23. Kiladze AA, Birkaia VG, Lomidze ES, Monaselidze DR: Different character of zinc ion binding with lactate dehydrogenase M4 in diluted and concentrated solutions. Biofizika. 41, 326–328 (1996)
24. Lecky FE, Little RA, Maycock PF et al.: Effect of alcohol on the lactate/pyruvate ratio of recently injured adults. Crit. Care Med. 30, 981–985 (2002)
25. Liber C: The metabolism of alcohol. Sci. Am. 234, 25–38 (1976)
26. Mao Y, Liu KJ, Jiang JJ, Shi X: Generation of reactive oxygen species by Co (II) from H_2O_2 in the presence of chelators in relation to DNA damage and Z'-deoxyguanosine hydroxylation. J. Toxicol. Environ. Health 47, 61–75 (1996)
27. Pesce AJ, Kaplan LA (1987): Method in Clinical Chemistry. Mosby Year Book, St. Louis.
28. Sarkola T, Iles MR, Kohlenberg-Mueller K, Eriksson CJ: Ethanol, acetaldehyde, acetate, and lactate levels after alcohol intake in white men and women: effect of 4-methylpyrazole. Alcohol. Clin. Exp. Res. 26, 239–245 (2002)
29. Shahangian S, Ash KO: Formic and lactic acidosis in a fatal case of methanol intoxication. Clin. Chem. 32, 395–397 (1986)
30. Smith SR, Smith SJ, Buckley BM: Combined formate and lactate acidosis in methanol poisoning. Lancet 8258, 1295–1296 (1981)
31. Tilton RC, Balows A, Hohnadel DC, Reiss RF (1996): Clinical Laboratory Medicine. Hohnadel D.C. Clinical Enzymology, Mosby Year Book, St. Louis, pp. 205–207.
32. Weintraub M, Stadnish R: 4-Methylpyrazole: an antidote for ethylene glycol and methanol intoxication. Hosp. Form. 23, 960–969 (1988)