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# In vitro study on lead and alcohol interaction and the inhibition of erythrocyte delta-aminolevulinic acid dehydratase in man[1]

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PRPIĆ-MAJIĆ D, TELIŠMAN S, KEŽIĆ S. In vitro study on lead and alcohol interaction and the inhibition of erythrocyte delta-aminolevulinic acid dehydratase in man. *Scand J Work Environ Health* 10 (1984) 235—238. The effect of lead (Pb) and ethanol (EtOH) interaction on the inhibition of erythrocyte δ-aminolevulinic acid dehydratase (ALAD) was investigated in human blood in vitro. Two different doses of ethanol (equivalent to 16.28 mmol of EtOH/l of blood and 108.53 mmol of EtOH/l of blood) and lead (equivalent to 2.17 μmol of Pb/l of blood and 4.34 μmol of Pb/l of blood) were examined separately and in combination. The dose-effect (EtOH-ALAD) relationship for a wide range of ethanol concentrations (0—217.06 mmol of EtOH/l of blood) was also investigated. The results obtained indicate that ethanol by itself does not inhibit ALAD, while lead does it readily. Neither ethanol concentrations significantly altered ALAD activity. The dose-effect (EtOH-ALAD) relationship did not reveal any inhibitory effect of ethanol on ALAD either; however, a weak trend towards increased ALAD activity was found. The effect of ethanol combined with lead indicated no significant difference as compared to the effect of the same dose of lead per se; however, a weak trend towards decreased ALAD activity was found. These findings support the hypothesis that the effect of ethanol on the transient inhibition of ALAD activity in vivo does not occur directly, but possibly through the intermediary action of lead from the body lead pool.

*Key terms:* blood lead, ethanol, total body lead pool.

An inhibitory effect of alcohol intake on the lead-sensitive enzyme (2) erythrocyte δ-aminolevulinic acid dehydratase (ALAD) has been reported for chronic alcoholics (6, 10, 11, 13, 14, 15) and after hard liquor ingestion (12, 14). Only in some studies (11, 13, 15) were blood lead data recorded, allowing for the establishment of the relationship between ALAD inhibition and blood lead and/or blood alcohol. The depression of ALAD by ethanol (EtOH) was explained by an increase in the intracellular redox potential through ethanol ingestion, the consequence being excessive concentrations of the sulfhydryl cofactor responsible for inhibiting enzyme activity (14). Danish authors (11) suggested that the depressing effect of alcohol intake on ALAD activity in chronic alcoholics (suffering from liver cirrhosis) occurred through the lead released from the liver into the blood due to acute alcohol-induced liver damage. Since ethanol intake increases the tissue redox potential in the organism [Both ethanol oxidizing to acetaldehyde and acetaldehyde oxidizing to acetate produce an equivalent of reduced nicotinamide adenine dinucleotide (NADH) and hydrogen ($H^+$).], it seems logical to suppose that alterations in the redox potential may also affect the redistribution of lead from the body lead pool and thus increase the biologically active lead fraction which in turn inhibits ALAD activity. This hypothesis was the basis for studies involving both in vitro and in vivo investigations. This paper deals with the results of the in vitro study.

## Materials and methods

The effects of two different doses (low and high) of ethanol and of lead in blood on ALAD activity were examined separately and in combination. The ethanol doses were equivalent to the concentration of 16.28 mmol of EtOH/l of blood (which produces a slightly drunken state) and of 108.53 mmol of EtOH/l of blood (a lethal dose). The lead doses were equivalent to an increase of 2.17 μmol/l (a moderately increased lead exposure) and of 4.34 μmol/l (a high lead exposure) in the blood lead concentration.

Three sets (A, B, C) of blood samples from normal healthy subjects nonoccupationally exposed to lead were collected with heparin as an anticoagulant. The subjects consumed no alcohol during the preceding 48 h, and the concentration of lead in blood and/or

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ALAD activity indicated a "normal" level of environmental lead exposure. Set A consisted of seven different blood samples, and sets B and C of six different blood samples each. The samples were divided into four (sets A and B) or five (set C) aliquots of 2 ml of blood in each tube. To the first tube (control) in each set of blood samples, 100 μl of deionized water was added. To the remaining tubes of each blood sample a total volume of 100 μl of ethanol-containing, lead-containing, or both ethanol- and lead-containing solutions were added. The ethanol used for preparing the solutions was from Merck (Uvasol, for spectroscopy), and the lead used was lead nitrate "BDH" (for atomic absorption spectroscopy).

In each set, prepared blood samples from each subject were preincubated at the same time in Parafilm-covered tubes at 37°C for 30 min before the ALAD determination (to simulate the in vivo conditions). A preliminary experiment examining different preincubation lengths (30, 60, 120, & 180 min) showed the lead-induced inhibition of ALAD to be dependent on the length of the preincubation time (the negative exponential relationship), while the ethanol effect on ALAD activity proved to be uninfluenced by the length of the preincubation period (ie, as compared to control blood samples which contained an equal volume of deionized water). Since the difference between the lead and the ethanol effect on ALAD activity was sufficiently pronounced during the preincubation period of 30 min (37°C), this time was chosen as the preincubation period for all the blood samples.

The dose-effect relationship between different ethanol concentrations (corresponding to 0—217.06 mmol of EtOH/l of blood) and ALAD activity was examined in four samples of heparinized blood, taken from four subjects nonoccupationally exposed to lead. Each sample was divided into six aliquots of 2 ml of blood. To the first tube (control) 100 μl of deionized water was added. To the remaining tubes 100 μl of ethanol-containing solution was added. Before the ALAD analyses the samples containing different ethanol concentrations (ie, 0, 16.28, 32.56, 65.12, 108.53 and 217.06 mmol/l of blood) were preincubated in Parafilm-covered tubes at 37°C for 30 min.

ALAD activity was measured by the standardized European method (1) (coefficient of variation ≤2 %). All analyses were performed in duplicate, and the mean values were used for the calculations. The results obtained are expressed by the means and standard deviations and compared with the corresponding control samples. In addition, the samples containing both ethanol and lead were compared with the matching samples containing only lead. The significance of the difference between the results was calculated by the Student's t-test (ie, $t = \bar{X}_{diff}/SE_{diff}$).

## Results

A comparison of the results in table 1, obtained after the addition of ethanol- and/or lead-containing solutions with respect to the control samples (containing an equal amount of water) showed that ALAD activity was significantly decreased in the blood samples containing only lead, or both lead and ethanol, while it was practically unchanged in the samples containing only ethanol. In the samples containing both ethanol and lead, a highly significant ($p < 0.001$) decrease in the ALAD values was found as compared with the corresponding control samples. The difference between these results and those from blood samples containing only lead was not significant. Indirectly, this result is proof that ALAD inhibition in the samples with both ethanol and lead was not caused by ethanol, but by lead.

The dose-effect relationship between the rising ethanol concentration added to human blood and the ALAD activity is shown in figure 1. The change in ALAD was minimal and the trend for the mean values

**Table 1.** The erythrocyte δ-aminolevulinic acid dehydratase (ALAD) activity in the control blood samples and the differences in ALAD due to the addition of ethanol (EtOH) and/or lead (Pb) with respect to the control samples and with respect to the addition of Pb per se.

| Set of samples | ALAD/nmol · s$^{-1}$ (l Ercs)$^{-1}$ (control value) | | EtOH and/or Pb added in blood | Difference in ALAD/nmol · s$^{-1}$ (l Ercs)$^{-1}$ | | | |
|---|---|---|---|---|---|---|---|
| | | | | With respect to control values | | With respect to Pb per se | |
| | Mean | SD | | Mean | SD | Mean | SD |
| A (N = 7) | 636.63 | 102.35 | 16.28 mmol EtOH/l blood | 10.17 | 12.34 | | |
| | | | 2.17 μmol Pb/l blood | −433.59* | 61.18 | | |
| | | | (16.28 mmol EtOH + 2.17 μmol Pb)/l blood | −433.92* | 60.18 | −0.17 | 10.50 |
| B (N = 6) | 598.79 | 132.69 | 108.53 mmol EtOH/l blood | 6.67 | 16.00 | | |
| | | | 2.17 μmol Pb/l blood | −341.23* | 57.34 | | |
| | | | (108.53 mmol EtOH + 2.17 μmol Pb)/l blood | −350.40* | 90.35 | −9.17 | 43.51 |
| C (N = 6) | 750.65 | 161.03 | 16.28 mmol EtOH/l blood | −13.34 | 39.17 | | |
| | | | 4.34 μmol Pb/l blood | −647.96* | 179.87 | | |
| | | | (16.28 mmol EtOH + 4.34 μmol Pb)/l blood | −652.30* | 172.70 | −4.50 | 20.67 |
| | | | (108.53 mmol EtOH + 4.34 μmol Pb)/l blood | −661.30* | 185.20 | −15.34 | 44.68 |

* $p < 0.001$.

was not decreasing, but rather increasing. This finding also supports our assumption that ethanol by itself does not inhibit ALAD activity in human blood.

## Discussion

Within the limits of the experimental conditions in the present study, the data obtained indicate no significant effect of ethanol per se on ALAD activity in human blood in vitro (table 1). The dose-effect relationship between ethanol and ALAD indicated no significant decrease in ALAD activity with respect to the increase of blood ethanol concentrations (figure 1). Moreover, a trend of *increasing* ALAD activity was observed. In addition the influence of ethanol combined with lead indicated no significant increase in ALAD activity, as compared to the effect of the same dose of lead per se (table 1). Moreover, a trend of *decreasing* ALAD activity was observed. Both these findings are in contradiction with the data reported by other authors who found a decrease in ALAD activity due to ethanol per se (14) and a depression of ALAD by both ethanol and lead, separately, that was partially reversed when the two were combined (13). However it is possible that at least part of this disagreement could be ascribed to different experimental conditions. It should be emphasized that the present in vitro experiment was performed with thoroughly washed, ie, lead-free, glassware.

Some of the reported conclusions on an "alcohol-induced" inhibition of ALAD activity are based on results from in vivo and in vitro experiments with rats (13, 14). However, the rat is not a suitable animal model for the investigation of effects which may occur in man under the influence of a toxic substance, particularly if these effects are mediated through oxido-redox alterations of the relevant enzymes. Ascorbic acid (a "vitamin" for man but not for rat), an important redox catalyzer, is a good example of this situation (3). The other example is glutathione. The level of erythrocyte glutathione is much higher in the rat than in man (5). The glutathione turnover in erythrocytes is also much higher in rat than in man (9). Moreover, for comparable blood lead levels, the erythrocyte ALAD activity in a "normal" rat is considerably lower than in a "normal" man (13). On the other hand, considerably higher blood lead levels are necessary to produce a reduction of approximately 50 % of the "normal" erythrocyte ALAD activity in rat, as compared to in man (13). These examples indicate that the intensity and dynamic pattern of changes in the oxido-redox system may differ significantly in rat and in man, thus the results should be interpreted with caution.

In the in vitro system of rat liver homogenate, a linear fall of ALAD activity with a rising ethanol concentration was found (13). In another paper, in an in vivo experiment with rats fasted for 48-h, 43.41 mmol of EtOH/kg of body weight (given orally) did not inhibit ALAD activity in the liver (7), and no significant change in ALAD activity could be observed during the 24 h following ethanol ingestion. This disagreement could probably be explained by the instability of the liver homogenate. Jocelyn (8) reported a rapid fall of the glutathione concentration in rat liver homogenate during incubation under aerobic conditions. Since ALAD is an enzyme which depends on glutathione concentration (4) for activity, the question arises as to whether the "alcohol-induced" inhibition of ALAD activity in vitro in the liver homogenate system is really due to the effect of ethanol only or is a reflection of glutathione variation in such systems. Since absolute anaerobic experimental conditions are extremely difficult to achieve, the question is pertinent also for anaerobic experimental conditions.

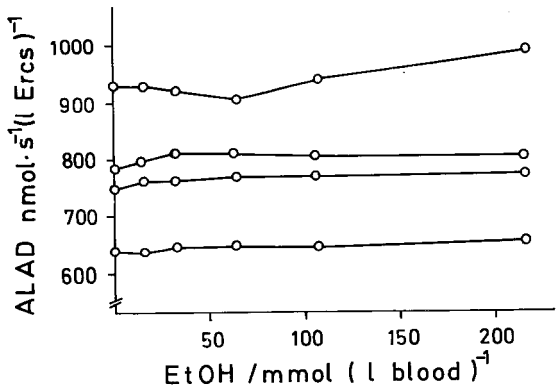


**Figure 1.** Dose-effect relationship between ethanol (EtOH) and erythrocyte δ-aminolevulinic acid dehydratase (ALAD) activity in vitro in blood samples of four "normal" subjects nonoccupationally exposed to lead.

The results of the present in vitro study suggest the possible involvement of lead in the inhibition of ALAD activity in vivo after alcohol ingestion. Before any conclusion can be drawn, an in vivo investigation should be carried out to complete the present study. However, the results of the in vitro study, even without the in vivo results, undoubtedly show that ethanol by itself does not inhibit ALAD and that lead does it readily.

## References


1. Berlin A, Schaller KH. European standardized method for the determination of δ-aminolevulinic acid dehydratase activity in blood. Z klin Chem klin Biochem 12 (1974) 389—390.
2. Bonsignore D, Calissano P, Cartasegna C. Erythrocytic ALA dehydratase and lead. Panminerva Med 8 (1966) 282—284.
3. Chatterjel IB, Kar IVC, Ghosh NC, Guha BC. Aspects of ascorbic acid biosynthesis in animals. Ann NY Acad Sci 92 (1961) 36—56.
4. De Barreiro OLC. Effect of sulfhydryl groups on 5-aminolevulinate dehydratase activity. Biochim Biophys Acta 178 (1969) 412—415.

5. Glock GE. Glutathione. In: Long S, ed. Biochemist's handbook. Spon EFN Ltd, London 1961, pp 793—795.
6. Hamlyn AN, Hopper JC, Skillen AW. Assessment of erythrocyte δ-aminolevulinate dehydratase for outpatient detection of alcohol liver disease: Comparison with γ-glutamyltransferase and casual blood ethanol. Clin Chem Acta 95 (1979) 453—459.
7. Held H. Die Wirkung von Äthylalkohol auf die Häm- und Porphyrinsynthese in der Rattenleber. In: Schlegel B, ed. Verhandlungen der Deutschen Gesellschaft für Innere Medizin, Achtzigster Kongres, Wiesbaden, April 21—25, 1979. Bergmann, München 1974, pp 481—484.
8. Jocelyn PC. The enzymatic oxidation of glutathione in rat liver homogenates. Biochem J 117 (1970) 947—949.
9. Jocelyn PC. Biochemistry of the SH groups. Academic Press, London, New York, NY 1972, pp 175—178.
10. Krasner N, Moore MR, Thompson GG, McIntosh W, Goldberg A. Depression of erythrocyte δ-aminolævulinic acid dehydratase activity in alcoholics. Clin Sci Mol Med 46 (1974) 415—418.
11. Magid E, Hilden M. Elevated levels of blood lead in alcoholic liver disease. Int Arch Occup Health 35 (1975) 61—65.
12. McColl KL, Thompson GG, Moore MR, Goldberg A. Acute ethanol ingestion and haem biosynthesis in healthy subjects. Eur J Clin Invest 10 (1980) 107—112.
13. Moore MR. Lead ethanol and δ-aminolævulinic acid dehydratase. In: Commission of the European Communities. Proceedings of the international symposium on recent advances in the assessment of the health effects of environmental pollution, Paris, June 24—28, 1974. Volume II. Luxembourgh 1975, pp 1171—1176.
14. Moore MR, Beattie AD, Thompson GG, Goldberg A. Depression of δ-aminolævulinic acid dehydratase activity by ethanol in man and rat. Clin Sci 40 (1971) 81—88.
15. Vives JF, Bellet H, Lapinski H, Miroouze D, Richard JL, Hirch JL, Soulayrac M, Mathieu-Daude P, Vallat G, Michel H. Alcoolisme chronique et intoxication saturnine. Gastroenterol Clin Biol 4 (1980) 119—122.

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